University of Alberta

Maternal Hypothyroxinemia, Fetal Growth Restriction, and Early Neonatal Morbidity

by

Michele Hamm

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_____
Dr Igor Burstyn

_____
Dr Nicola Cherry

_____
Dr Jonathan Martin

_____
Dr Jeremy Beach

Abstract

Maternal thyroid function is closely tied with normal fetal developmental processes, and clinical thyroid hormone deficiencies have been associated with a number of adverse outcomes, ranging from obstetric complications to impaired neurodevelopment. Maternal hypothyroxinemia, a subclinical thyroid hormone deficiency, has also recently been proposed to be detrimental in fetal development. To determine whether this condition is adversely related to fetal growth or early neonatal morbidity, a prospective cohort was employed to examine the association between maternal hypothyroxinemia during the early second trimester of pregnancy and the outcomes of fetal growth restriction and neonatal condition at birth. Using measures of small for gestational age, standardized birth weight z-score, and low Apgar score, no relationship was found between a maternal thyroxine deficiency and a compromised neonatal presentation.

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List of Abbreviations

AGA	Appropriate for gestational age
APHP	Alberta Perinatal Health Program
CI	Confidence interval
CNS	Central nervous system
D1	Type I iodothyronine deiodinase
D2	Type II iodothyronine deiodinase
D3	Type III iodothyronine deiodinase
DIT	Diiodotyrosine
fT_4	Free thyroxine
GH	Growth hormone
GLUT1	Glucose transporter 1
GTT	Gestational transient thyrotoxicosis
hCG	Human chorionic gonadotropin
HTN	Hypertension
IGF	Insulin-like growth factor
IQ	Intelligence quotient
IQR	Interquartile range
IUGR	Intrauterine growth restriction
LOD	Level of detection
MCT8	Monocarboxylate transporter 8
MIT	Monoiodotyrosine
mRNA	Messenger ribonucleic acid
NBAS	Neonatal Behavioral Assessment Scale
NHANES	National Health and Nutrition Examination Survey
PBB	Polybrominated biphenyl
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
PDI	Bayley Psychomotor Developmental Index
PFA	Perfluorinated acid

PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
RR	Relative risk
rT_3	Reverse triiodothyronine
SD	Standard deviation
SE	Standard error
SGA	Small for gestational age
T_2	3,3'-diiodothyronine
T_3	Triiodothyronine
T_4	Thyroxine
TBG	Thyroxine-binding globulin
TETRAC	Tetraiodothyroacetic acid
TPO-Ab	Thyroid peroxidase antibody
TR	Thyroid receptor
TRH	Thyrotropin-releasing hormone
TSH	Thyroid stimulating hormone (thyrotropin)
UCP1	Uncoupling protein
WHO	World Health Organization

Chapter 1
Introduction

1.1. Literature review

1.1.1. Background

The course of fetal growth is highly significant in determining neonatal well-being and subsequent postnatal development. The impact of a restriction of fetal growth can be keenly felt, as these infants have an increased susceptibility to immediate and long-term morbidities, as well as a greater risk of mortality (1). The etiology of fetal growth restriction is complex, and currently there exists a plethora of known and suspected risk factors. However, in many cases, the details of the underlying mechanisms associated with these risk factors remain largely speculative. A reduced ability to transfer nutrients and oxygen to the fetus is one of the dominant proposed pathways in the etiology of fetal growth restriction, potentially arising as a result of decreased uterine blood flow, placental insufficiency, or maternal malnutrition. Disruptions of crucial signaling molecules are also believed to be important, with evidence supporting an important role of the availability of hormones such as insulin, thyroxine, and glucocorticoids, as well as other factors such as insulin-like growth factors (2).

Hormonal regulation is a key process in fetal development. Organogenesis is highly dependent on the precise coordination of chemical signals according to a strict temporal sequence, and cues from thyroid hormones are among those that promote development, being key contributors to normal cardiac, pulmonary, and nervous system functioning (3). In 1999, two publications were released suggesting that disruptions of maternal thyroid homeostasis can disturb fetal development, despite the normal functioning of the fetal thyroid gland. Impaired fetal neurodevelopment was found to be associated with both maternal hypothyroidism (4) and maternal hypothyroxinemia (5), a condition widely regarded as a subclinical thyroid hormone deficiency, in which free thyroxine levels are reduced and thyroid-stimulating hormone concentrations remain in the normal range. In response to the study on hypothyroidism (4), a letter to the editor suggesting that

hypothyroxinemic women in the control group could be diluting the effect size of the adverse neurodevelopmental effects associated with hypothyroidism prompted further analysis (6). In a regression examining maternal thyroxine levels and the neurodevelopment of the children in the control group, a 10-point increase in IQ was observed from the low to the high ends of the range of free thyroxine levels ($p=0.13$), a trend supporting the claim that hypothyroxinemia impairs fetal development in a manner similar to that of hypothyroidism (6). These studies raised the profile of research on hypothyroxinemia, focusing more attention on the effects of this condition on perinatal outcomes, including on fetal growth restriction (7-9).

This chapter will be organized as follows: a brief overview of fetal growth restriction and early neonatal morbidity will be provided, followed by a description of the thyroid gland and its role in pregnancy and development. Selected thyroid diseases will be examined, with a focus on hypothyroxinemia and its relationship to fetal growth.

1.1.2. Restriction of fetal growth

Classically, fetal growth restriction has been measured as low birth weight. According to the World Health Organization (WHO), an infant with a low birth weight is one less than 2,500 grams at its first weighing, which is ideally performed within one hour of birth (10). This has been consistently shown to be an important predictor of neonatal morbidity and mortality and is currently in widespread use. While birth weight is easily obtained and measured, low birth weight in and of itself does not accurately describe the well-being of the newborn. Two important factors to consider in the interpretation of birth weight are the length of gestation and the presence of intrauterine growth restriction.

Length of gestation is inherently variable among births in a population, with deliveries between 37 and 42 weeks being term deliveries, and those prior to 37 weeks being preterm (11). In Alberta, the Canadian province where this research is set, this is an issue of considerable concern, with 3881 of the 42,019 live births in 2005 occurring prematurely (9.2%) (12). The vast majority of fetal growth occurs in the second half of pregnancy, with up to 95% of an infant's weight being gained during this time (13). If

the duration of gestation is interrupted, it follows that fetal growth and development may not have reached their optimal levels. Premature delivery is often associated with a reduced birth weight relative to the mean of all births, as well as a number of morbidities, such as respiratory distress syndrome (1). The risk of mortality is also greatly increased among preterm infants: 32.6% of neonatal deaths in Canada can be attributed to premature birth (14). In 2005, 74% of all low birth weight births in Alberta were the result of preterm delivery (2030/2726) (14). While this represents the most significant cause of low birth weight, premature delivery is associated with a set of distinct risk factors, and these infants are not necessarily subject to an impaired rate of growth. The most recent figures available for Alberta data suggest that 46% of preterm infants are born weighing more than 2,499 grams (1745/3775) (14).

The second major contributor to low birth weight is intrauterine growth restriction (IUGR). This is a measure of the inability of an infant to attain its full growth potential prior to parturition (15). IUGR can arise as a maladaptive response to various underlying pathologies, including a number of genetic and environmental factors (16). Commonly, it is defined as a birth weight below the tenth percentile according to gestational age and infant gender (16). Despite the recognition that individual fetal growth is programmed to an extent by certain factors such as maternal race, height, and parity (17), and can be impeded by biological, chemical, and physical elements, growth potential is not easily measured in a clinical context, due to varying growth trajectories (18). The best prenatal estimation of IUGR occurs through the use of at least two successive ultrasound measurements taken two to four weeks apart to gain an approximation of the rate of fetal growth. The progression and severity of growth restriction can then be monitored, allowing for the best possible confirmation of IUGR (19). Due to the difficulties in measuring fetal growth restriction, the term small for gestational age (SGA) is often used as a proxy in population research, although not in clinical practice.

Small for gestational age is a statistical definition used for the classification of infants with a birth weight below a set percentile, usually the tenth, for their gestational age and gender (20). This is a term that is used solely to describe the size of the infant at birth,

without considering the degree or rate of fetal growth *in utero* (21). While the shared use of the tenth percentile cut-off for both IUGR and SGA implies that the two conditions are synonymous, they are distinct entities. Overlap between the two is possible, although not necessary (21), and it is often assumed that an infant presenting as SGA will have suffered from IUGR.

This assumption has led to a number of discrepancies and inconsistencies in the literature. The terms IUGR and SGA are often used interchangeably, occasionally without provision of an explicit definition. Additionally, the statistical limits used to define SGA are not universally maintained at 10 percent. Depending on the source, the third, fifth, or tenth percentile may be used, or a point 2 standard deviations below the mean (17). Another interesting consideration is that the use of SGA as a measure of IUGR may not have the same relevance when used for preterm infants as for full-term infants (22). As the birth weight references used in the classification of SGA are based only on live births, it is likely that at earlier gestational ages, they also capture the influence of underlying factors that are associated with prematurity. In this way, the birth weights provided to calculate SGA are more reflective of the induction of preterm delivery than of growth restriction, and the smallest 10% of the live born infants will be much smaller than the smallest 10% of live births combined with those remaining in utero.

Given the wide range of variability in the usage of these commonly measured end-points, it is essential to remain mindful of the context in which the terms SGA and IUGR are used for meaningful interpretations to be made. Throughout this manuscript, SGA infants will be defined as those in the lowest tenth percentile according to gender and gestational age, and will be used as a surrogate for IUGR.

1.1.3. Early neonatal morbidity

The condition of a newborn at the time of delivery can be strongly predictive of neonatal morbidity and mortality. In 1952, a scoring system was proposed by Virginia Apgar to rapidly evaluate the condition of the infant and its response to resuscitation (23). This scale has remained clinically relevant and is in widespread use today. The Apgar score is

based on five parameters: heart rate, respiratory effort, muscle tone, reflex irritability, and color, and each is scored from 0 to 2 for a maximum score of 10 (Table 1.1). Scores of 7 or higher are considered normal, 4-6 are intermediate, and below 3 are low (24). Apgar scores are recorded at 1 and 5 minutes after birth, with the 5-minute score better reflecting the likelihood of survival (25). In a recent study of 132,228 singleton term deliveries, it was found that neonatal mortality was highly correlated with Apgar scores below 7, with a relative risk (RR) of 1460 (95% confidence interval (CI) 835 to 2555) for scores 0-3, and a RR of 53 (95% CI 20 to 140) for scores 4-6, when compared to infants with scores 7-10 (26).

While the Apgar score has been shown to have merit as a predictor of neonatal mortality in term infants, it is inappropriate for use in the prediction of neurologic outcome or in the diagnosis of asphyxia (24). These can both be associated with a low Apgar score, but there are numerous other factors that can result in a depressed score independently of these outcomes. Included among these are the maternal use of analgesics or sedatives, airway obstruction, and physical immaturity (27) (Table 1.2). Physical immaturity is highly relevant due to its association with preterm delivery. Premature infants often have low Apgar scores due to underdevelopment, despite being otherwise healthy. For this reason, the score's significance as a predictor of mortality is limited among premature births, and should only be used as an assessment of the infant's condition in the delivery room to guide the need for resuscitation (24).

While there are limitations to its use, the Apgar score remains an important measure of a newborn's physical condition and the five minute score will be used in this thesis as an indicator of early neonatal morbidity.

1.1.4. Thyroid hormone homeostasis

1.1.4.1. Function of the thyroid gland

The thyroid gland plays a critical role, acting to maintain metabolic homeostasis in the body (28). Its influence is far-reaching, with nearly all physiological systems impacted to some degree. Through the secretion of its two major hormones, thyroxine (T_4) and

triiodothyronine (T_3), the thyroid is able to regulate cardiac, gastrointestinal, and kidney function; manipulate energy usage, heat generation, and weight; and play an essential role in early fetal and childhood growth and development (29) (Table 1.3). These actions are generally enabled by the stimulation of lipid and carbohydrate metabolism, which in turn translates into oxygen consumption and action at the level of the target tissues (28). The few metabolically active tissues to escape input from the thyroid are the adult brain, testes, uterus, lymph nodes, spleen, and anterior pituitary (28). While the thyroid gland is not essential for survival, its absence is associated with poor mental and physical performance, an impaired ability to withstand cold, and mental retardation and stunted growth (dwarfism) in children (28).

Of the two major hormones secreted by the thyroid, T_3 is the more biologically active species, being three to five times more potent than T_4 (29). Despite its higher level of activity, the concentrations of T_3 released into the circulation are far surpassed by those of T_4. Approximately 80μg (103nmol) of T_4 is released from the thyroid per day, compared to 4μg (7nmol) of T_3 (28). Additionally, 2μg (3.5nmol) of the biologically inactive reverse triiodothyronine (rT_3) is secreted (28). Once secreted from the thyroid, approximately one-third of the available T_4 is converted to T_3, increasing the amount of accessible substrate necessary for biological activity, while 45% is transformed to rT_3 to eventually be converted to the weakly active diiodothyronines, and the remainder is largely plasma protein bound (28) (Figure 1.1). The fraction of hormones that remain unbound make up the active species, and free T_3, as well as free T_4 to a limited extent, are able to bind to the hormone-sensitive nuclear transcription factors that act as thyroid hormone receptors (TRs) in their target tissues, thereby exerting their actions (28).

The most established mechanism of regulation of thyroid hormone activity comes in the form of a classical endocrine negative feedback loop (Figure 1.2). This is initiated at the level of the hypothalamus, in which thyrotropin-releasing hormone (TRH) is produced (29). TRH travels to the thyrotropic cells in the anterior pituitary, stimulating the production and secretion of thyroid-stimulating hormone (TSH; formerly called thyrotropin) (29). TSH acts on the thyroid gland, promoting the production of T_3 and T_4

(29). Once T_3 and T_4 are released into the bloodstream, the free fractions of each are responsible for providing feedback to the hypothalamus and pituitary to either stimulate or inhibit further TRH and TSH signaling. This feedback occurs largely at the level of the pituitary, and is primarily the result of regulation by free T_3 rather than free T_4 (28).

One of the most important aspects in determining the necessary levels of thyroid stimulation is the level of available iodine. Iodine is required for the synthesis of thyroid hormones, being a fundamental element of the building blocks monoiodotyrosine (MIT) and diiodotyrosine (DIT). Under normal conditions, one molecule of MIT and one of DIT will condense to form T_3, while two molecules of DIT will join to form T_4 (28). However, in the case of an inadequate supply of iodine, these precursor molecules cannot be synthesized in sufficient amounts, thereby limiting the amount of thyroid hormones produced. In this event, MIT is preferentially produced over DIT, resulting in a greater rate of production of T_3 than T_4 (29). With reduced amounts of hormones in circulation, TRH, and especially TSH, secretion is increased in an attempt to compensate for this deficiency. Conversely, when the intake of iodine is excessive, TSH secretion is inhibited due to sufficient levels of thyroid hormone production (29). It has been observed in situations of mild to moderate iodine deficiency that the thyroid has the ability to maintain euthyroid status through a number of autoregulatory mechanisms that do not require the stimulatory effects of the pituitary and TSH (30). Among these mechanisms are increased thyroidal blood flow and volume, increased thyroidal iodine clearance, increased half-life of iodine-containing compounds, and preferential synthesis of T_3 over T_4.

Another regulatory factor involved in the maintenance of appropriate circulatory thyroid hormone concentrations is the extensive plasma protein binding that occurs with these hormones. It is estimated that more than 99% of both T_4 and T_3 are bound to plasma proteins (28). T_3 and T_4 are able to bind to three proteins: thyroxine binding globulin (TBG), transthyretin, and albumin. By far, TBG carries the greatest quantities of thyroid hormones, with approximately 70% of the circulating amounts of both T_3 and T_4 being held by this protein (29). Transthyretin acts as a ready supply of T_4, having a fairly high

affinity for the hormone, as well as engaging in rapid dissociation (29). Albumin has the lowest affinity of the three plasma proteins for thyroid hormones, but as it is present in such high quantities in the bloodstream, it is still responsible for transporting 15% of the hormones in circulation (29). Like transthyretin, albumin is associated with rapid dissociation rates, and therefore acts as an easily accessible store of both T_3 and T_4 (29). The prominent role of plasma proteins in thyroid hormone homeostasis has a number of advantages: it enables the transport of hormones that have poor solubility in water, it makes available large quantities of the hormones, and it facilitates the even distribution of the hormones among their target tissues (29).

The degradation of thyroid hormones is carried out by the deiodinase family of enzymes. There are three types of deiodinases: D1, D2, and D3 (also known as types I, II, and III, respectively) (31). D1 is found in the liver, kidney, muscle, and thyroid and its role is to transform T_4 to T_3 and rT_3 to the similarly inactive 3,3'-diiodothyronine (T_2) (28). D2 acts as the major activating enzyme, converting T_4 to T_3, while D3 is the major deactivating enzyme, converting T_4 to rT_3, and T_3 to T_2 (29,31). Approximately 80% of T_4 is degraded via deiodination, with its metabolites including both T_3 and rT_3 (29). It is through this process that the majority of T_3 is produced (29).

1.1.4.2. Maternal thyroid function during pregnancy

During pregnancy, the demands on the maternal thyroid gland are greatly increased. Not only do a number of physiological changes occur that affect thyroid function (for example, increased estrogen concentrations stimulate TBG production (32)), but the mother is also required to provide adequate amounts of thyroid hormones both for herself and for the developing fetus. This is achieved through a number of hormonal alterations. One of the major influences comes in the form of the previously alluded to increase in the concentration of TBG. Throughout the first trimester, the levels of TBG rise until they reach a plateau at approximately 12-14 weeks of gestation (33). With a greater availability of plasma proteins, thyroid hormone binding increases, resulting in the stimulation of increased secretion of T_4 and T_3. Therefore, the levels of total T_3 and T_4 increase in parallel with those of TBG (33). Also contributing to the rise in total thyroid

hormone levels is the presence of human chorionic gonadotropin (hCG), a hormone that is secreted by the placenta following implantation of the newly conceived embryo (11). The α-subunit of hCG shares structural similarities with that of TSH, and the two proteins are in fact close enough that hCG can stimulate thyroid hormone receptors (34). In this manner, hCG seems to transiently take on the role of TSH, and as it increases during the first trimester, TSH decreases accordingly (33). Finally, the levels of free T_4 and T_3 are also changed as a result of an exceptionally high level of deiodinase activity in the placenta (35). While the concentrations of both T_3 and T_4 show a temporary increase in early gestation, due to the actions of hCG and TBG, the wide distribution and high levels of D3 activity serve to inactivate these hormones to a greater extent than would occur in a non-pregnant state. In this manner, by the end of a woman's pregnancy, her free hormone levels are reduced to approximately 10-15% lower than those of a non-pregnant woman (33,35). Related to hormonal changes are the alterations in iodine demand and usage during pregnancy. While demand increases, due to increased hormone production (36), availability is reduced. This deficiency arises as a result of two phenomena: an increased renal iodine clearance (29,33,34), and maternal transfer of iodine to the fetus (33).

1.1.4.3. Thyroid function in fetal and infant development

The thyroid gland is a significant contributor to the course of fetal and infant development, playing a critical role in coordinating the maturation of the skeletal, auditory, visual, hepatic, pulmonary, and cardiac systems, as well as to the functionality of brown adipose tissue, and most importantly, the brain (37). Beginning even prior to the onset of fetal thyroid function, and up until approximately two years of postnatal life, neurological development is reliant on the input provided by thyroid hormones (37). Regulation of thyroid hormone signaling is multi-factorial, with control being exerted through the expression of thyroid hormone receptors and deiodinases, both at the maternal and fetal level, as well as through feedback mechanisms and hormone availability.

The beginnings of fetal thyroid functioning are marked by the appearance of thyroid hormone receptors at 8-10 weeks of gestation (30,36,37), the initiation of iodine trapping at approximately 11-12 weeks (29,35), and the subsequent secretion of TSH and T_4 around 12 weeks (34). By 18-20 weeks, the hypophysial portal system has developed (29,30), and the fetal thyroid becomes fully functional (35). However, up to this point, the fetus is largely reliant on a maternal supply of hormones. Placental permeability to thyroid hormones is limited due to extensive deiodinase activity (38). The inactivating enzyme D3 is highly expressed in the placenta, converting the majority of the available T_4 to rT_3 and T_3 to T_2 (29,31). Despite this activity, iodine and TRH are able to freely cross the placenta and small quantities of T_4 are able to pass into the fetal circulation, but any transfer of T_3 is negligible, and TSH does not cross at all (34,36). In this way, the mother acts as a source of thyroid hormones, both directly, through the limited provision of T_4, and indirectly, as T_4 can be converted by the fetus to the more active T_3 (30). Later on in development, once the fetal thyroid has begun to operate, the maternal supply of iodine enables hormone synthesis (34).

Once in the fetal circulation, thyroid hormones are preferentially used in the regulation of proper brain development (29). Precision is crucial, and to maintain appropriate levels of thyroid hormones, both D3 and the major activating enzyme D2 are expressed in the fetal brain, with activity being detected as early as 7-8 weeks of gestation (37). D2 is critical in making available the necessary T_3, through the conversion of T_4, while D3 is charged with preventing an excessive presence of thyroid hormones. Additionally, the thyroid hormone receptor isoforms TRα1, TRα2, and TRβ1 have been detected during the same timeframe, around 8 weeks of gestation (37). The activity of these enzymes and receptors so early in gestation suggests that fetal development does not occur independently of maternal thyroid activity, underscoring the importance of maternal thyroid homeostasis.

The second half of the gestational period is marked by increases in total and free T_4 and to a lesser extent, total and free T_3, TBG, and TSH (37). This corresponds to the latter half of cerebral neurogenesis and migration, as well as the initiation of neuronal

differentiation, synaptogenesis, and myelinogenesis, processes which extend into infancy and early childhood (37). Also of importance in early development is the impact of thyroid hormone mediation on growth and on nonshivering thermogenesis. Bone maturation depends both directly and indirectly on thyroid hormone availability, as T_3 regulates endochondral ossification and growth plate chondrocyte differentiation, as well as the expression of growth hormone and insulin-like growth factor (IGF) (37). At the time of birth, thyroid hormones also act in concert with catecholamines to stimulate the transcription of uncoupling protein (UCP1) in the mitochondria-rich cells of brown adipose tissue. The actions of UCP1 serve to release energy as heat in a reaction vital to the transition of the fetus from the maternal to the external environment (37).

1.1.4.4. Selected thyroid disorders during pregnancy

As described above, the influence of the thyroid is extensive in the normal development of the fetus. Being involved in such vital processes, it is not surprising that severe problems arise in the event of a disruption of thyroid hormone homeostasis.

1.1.4.4.1. Hypothyroidism

It has been well-documented that a deficiency in thyroid hormones during gestation, whether as a result of decreased maternal transfer, or of a fetal defect, can lead to impaired development in the central nervous system (CNS), with effects ranging from mental retardation to motor-rigidity to deaf-mutism (28). Hypothyroidism, which is defined by the presence of TSH levels greater than the 98th percentile of the general population (30), therefore implying a deficit of free T_4 and T_3, has long been associated with these conditions. Children with congenital hypothyroidism have been observed to display slowed bone growth, delayed epiphyseal closure, and a depression of growth hormone (GH) secretion (28). Neuropsychological performance has been shown to suffer (4,35), and congenital hypothyroidism is believed to be one of the most common causes of preventable mental retardation (28). Maternal hypothyroidism is associated with a number of additional adverse pregnancy outcomes, including miscarriage, preeclampsia, anemia, decreased birth weight and head circumference, placental abruption, fetal distress, and perinatal mortality (39,40). Although hypothyroidism is a preventable

condition, it still has a prevalence of approximately 1-2% in pregnant women, and another 2-3% of expecting mothers are diagnosed with subclinical hypothyroidism, with normal levels of T_3 and T_4 but elevated TSH (39).

Fetal growth restriction has been examined in the context of hypothyroidism, typically as one of many measures of overall fetal or neonatal wellbeing. It is only recently that the consequences of hypothyroidism on the neonate have been considered at all, since maternal thyroid dysfunction was long associated with anovulation (41). However, with more successful pregnancies being observed, the effects of both maternal and fetal thyroid function on the development of the fetus have been a source of interest.

The association between severe growth restriction and fetal thyroid hormone levels was examined in an early study conducted by Thorpe-Beeston and colleagues (42). Small for gestational age was diagnosed using abdominal circumference ≥ 2 standard deviations below the normal mean, as determined by ultrasound. Forty-nine SGA fetuses between 21 and 38 weeks gestation were selected from a group of infants that had been diagnosed with growth restriction in this manner and who were referred for karyotyping, following which their serum samples were collected by cordocentesis and were analyzed for TSH, total and free T_4 and T_3, and TBG levels. Fetal thyroid function was then assessed using these parameters, using reference ranges previously collected from a cohort of 62 appropriately grown fetuses (appropriate for gestational age; AGA) (43). To account for the expected changes in thyroid hormone levels over the course of pregnancy, comparisons were made between the SGA and AGA groups using the standard deviations from the mean for each of the outcome measures as per gestational age. It was found that the group of fetuses displaying SGA had significantly increased TSH levels (mean difference 1.24 SD; $p<0.0001$) and decreased total (mean difference -0.951 SD; $p<0.001$) and free T_4 (mean difference -1.218 SD; $p<0.0001$) levels when compared to the AGA group, suggesting fetal hypothyroidism. The use of standard deviations in the reporting of differences in hormone measurements was a strength in this study, as the fetal serum samples were collected from a wide range of gestational ages, making it impossible to directly compare thyroid hormone measurements. While this study does provide a

correlation with restricted fetal growth and diminished thyroid function, it is impossible to determine whether IUGR is the result, or the cause, of altered thyroid function. In their discussion, the authors suggest that a hypothyroid state is an adaptive, and even advantageous, function of the fetus in response to IUGR, as it results in a decrease in metabolic rate and oxygen consumption, allowing conservation of the resources supplied by the mother via the placenta. While this may indeed represent an adaptive state, it is no longer likely to be considered advantageous, with more recent evidence indicating that fetal thyroid hormone deficiency can permanently impair neurodevelopment and future intellectual functioning (4,36).

At the level of maternal hypothyroidism, evidence has suggested that birth weight is slightly decreased in response to even mild maternal thyroid dysfunction, for example, in the work by Glinoer *et al.* in 1991 (44). Selecting a cohort of 120 women who were clinically euthyroid, but who had mild thyroid abnormalities, the authors followed the women from a point early in gestation (>70% prior to 20 weeks) through to their deliveries. This population was heterogeneous, with the disorders suffered by cases including goiter, nodules, thyroid autoantibodies, and a history of a past thyroid disorder. Given that the population was selected from an area with moderate iodine deficiency, and that the past thyroid disorder subset included women who had previously been on thyroid hormone supplementation, this group could be considered to share similar features with hypothyroid women and will be discussed further. This group was comprised of 11 women who had TSH levels similar to those of the controls and a slightly elevated free T_4 concentration. While these parameters do not suggest hypothyroidism, the serum samples from these women were collected at an earlier point in gestation than those of controls (11 vs. 15 weeks; $p<0.03$), which is very likely to account for this discrepancy. Of the indices collected to measure fetal development, the only significant finding was that women with a past history of thyroid disease gave birth to infants with a slightly reduced birth weight compared to controls (2.9 kg, compared to 3.3 kg; $p<0.01$), while gestational age and hormonal parameters were not influenced. The evidence provided by this study is not particularly strong, notably due to the heterogeneity of the population and the small sample size. The classification of past thyroid disease is broad,

encompassing a number of different conditions with varying etiologies and expressions. However, it allowed for the closest extrapolation to women with hypothyroidism and was therefore not discounted in terms of providing preliminary trends. Another limitation is the lack of a documented, or adjusted for, date of collection of maternal serum samples. Thyroid hormone levels change over the gestational period, not necessarily allowing for the comparison of measurements taken at different time points. Finally, the measurement of birth weight is limited in its ability to be interpreted without being put in the context of gestational age, but the gestational age at delivery did not differ significantly between the past thyroid disease group and the control group (38 weeks vs. 39 weeks), thereby potentially negating this concern.

Even when treated, maternal hypothyroidism has been observed to have an impact on fetal growth. In a case control study of full-term infants born to healthy women (N=139), or to women with hypothyroidism treated with thyroxine supplementation (N=246), it was found that the birth weight and head circumference in the study group were significantly reduced when compared to the control group ($p<0.001$) (45). When limiting the analyses to infants born AGA, the results remained unchanged. This study provides an interesting perspective, since all mothers in the study group were being actively treated for a thyroid hormone deficiency. It is possible that the prescribed treatment was inadequate, which is plausible considering that TSH and free T_4 levels fluctuate over the course of the pregnancy, making it a difficult condition to control. If a relapse to a hypothyroid state were to occur during a critical window during fetal development, the insufficiency of thyroid hormones during that time could be translated into adverse developmental effects such as fetal growth restriction. This study had a number of strengths. While fetal growth was measured as birth weight, the study design limited subjects to full-term births, and further adjusted for infants that were appropriate for gestational age, with no resulting change in the reported results. It was also highly representative of its target population, including all relevant births from a single centre over the course of a six year period (January 1994 – December 1999). All infants born to thyroxine-supplemented hypothyroid women were potential subjects (N=259), with the only exclusions being infants born to mothers that already had a child during the study

period or who were second-born twins (N=9), and those with permanent congenital hypothyroidism (N=4). The control group was made up of all healthy infants born to healthy women who underwent at least two thyroid function tests during pregnancy with normal results. Interestingly, when fetal thyroid function was measured, it was found that the infants with the highest levels of TSH also had the lowest birth weights ($p<0.035$) and smallest head circumferences ($p<0.001$). The TSH levels of the neonates were found to demonstrate a positive correlation with maternal levels ($r=0.751$; $p<0.001$), suggesting that the severity of growth restriction is determined in a dose-dependent manner, corresponding to the severity of maternal hypothyroidism. The significance of the finding that head circumference was reduced is less clear, since typically, this is a finding associated with hyperthyroidism, while hypothyroidism is generally associated with an increase in this parameter (46,47). However, it was proposed by the authors that this could be the result of the variations in thyroid hormone availability – in times of insufficient maternal provision, the fetus may overcompensate with increased production of T_4, resulting in a transient hyperthyroid state.

In another study examining the effect of treatment of maternal hypothyroidism, as well as the timing, women with controlled hypothyroidism (N=127 during the first trimester; N=123 during the third trimester) were compared to women with uncontrolled hypothyroidism (N=40 during the first trimester; N=44 during the third trimester) in the context of neonatal and obstetric outcomes (48). While a trend towards a reduction in birth weight, as well as an increased risk of low birth weight was observed in the uncontrolled group at both time points, none of the observed associations reached significance (effect sizes not provided). It is unlikely that thyroid hormones are responsible for this trend however, since in all cases but one, low birth weight was due to premature delivery, negating any likelihood that thyroid hormone levels played a role. This study employed a retrospective design, using a database generated from patients presenting to an antenatal endocrine clinic. Thyroid hormone measurements were available, as well as certain obstetric and neonatal outcomes, such as birth weight and rate of Caesarean delivery, but there was no information available on any other potential confounding factors, making the applicability of these findings somewhat limited.

Additionally, while each of the outcomes was compared between women with controlled and uncontrolled hypothyroidism at two separate time points, it would have been interesting to compare the differences between the outcomes within the thyroid status classifications across time periods, an analysis that was not provided in this paper.

Birth weight is commonly reported as a neonatal measure in studies of hypothyroidism, but the evidence on fetal growth restriction as a primary outcome is limited. However, the trend towards a reduction in birth weight is fairly consistently seen across studies, and is frequently reported as an adverse outcome associated with hypothyroidism (39,49). Although birth weight is not the best measure of fetal growth restriction, it seems likely that a deficiency in thyroid hormones does lie on the causal pathway to this particular outcome.

Taken together, the adverse effects of hypothyroidism have encouraged recommendations for the implementation of universal prenatal screening programs from a number of endocrine societies, including the American Thyroid Association, The Endocrine Society, and the American Association of Clinical Endocrinologists (50-52). Measurements of serum TSH and free T_4 can be easily obtained, determining the appropriate course of treatment if necessary (53). Thyroxine therapy is very effective in the management of hypothyroidism, and is recommended for use throughout pregnancy, adjusting the dosage to accommodate the increased demands on thyroid hormone availability (54). Currently in Alberta, universal screening for thyroid disease among pregnant women is not in effect, with testing being limited to women with possible clinical features.

1.1.4.4.2. Hypothyroxinemia

Although the diagnostic feature of hypothyroidism is an elevated TSH concentration, there is speculation that the underlying cause of the consequences associated with this condition is a deficiency of T_4, regardless of TSH levels. Hypothyroxinemia is defined as a reduction in free T_4 levels to a point below a set cutoff (typically the 5^{th} or 10^{th} percentile) without the expected increase in TSH to accompany this drop (55). Initially this description appears to be counterintuitive, as thyroid hormone levels are subject to

negative feedback mechanisms that dictate that a reduction in free T_4 will be accompanied by a compensatory increase in TSH. However, as described above, the thyroid has a number of autoregulatory mechanisms that allow it to normalize TSH levels in the context of a T_4 deficiency, including the preferential synthesis of T_3 over T_4 (30). This response suggests that while the production of T_4 may suffer, T_3 is still synthesized at a normal, or even an increased rate, thereby maintaining TSH homeostasis and preventing the expected increase in concentrations (36). Therefore, while the increased levels of free T_3 in the maternal circulation may be sufficient to maintain the mother's requirements, the deficiency in free T_4 can still be experienced by the fetus in the absence of overt maternal symptoms, as T_3 is unable to cross the placenta. In addition to the presence of autoregulatory mechanisms, the thyroid hormone imbalance may be the result of improper signaling from the hypothalamic-pituitary-thyroid axis, or dysfunction at any of those levels. With the recognition that this phenomenon exists, several animal and epidemiological studies are reporting findings that support the suggestion that the adverse neurodevelopmental outcomes seen in cases of hypothyroidism can actually be attributed to reduced levels of free T_4, whether or not the levels of TSH are augmented (30).

Much of the work in the area of hypothyroxinemia has been conducted by the research teams of Dr Victor J. Pop (Tilburg, the Netherlands). In 1999, Pop *et al.* initiated a series of studies that suggested that low levels of maternal free T_4 in women without overt thyroid dysfunction are associated with impaired fetal and infant neurodevelopment (5). In the first study, a cohort of 220 healthy infants born to women without thyroid disease were assessed for neurodevelopment at 10 months of age using the Bayley Scales of Infant Development. The study group was limited to women receiving antenatal care at 12 weeks gestation from one of five centers in Veldhoven, the Netherlands, between January and November 1994. Any women receiving thyroid medications were excluded, as were any women experiencing pregnancy-related complications. Infants were limited to those that were singletons, born at term, and without evidence of growth restriction or congenital anomalies, including hypothyroidism. Serial measurements of maternal thyroid hormone levels were taken at 12 and 32 weeks gestation, as well as at 4 weeks

postpartum, and at 8 week intervals until 36 weeks postpartum. When looking at women with free thyroxine levels below the 10th percentile (10.4 pmol/L) at 12 weeks gestation, the investigators found that these infants (N=22) had significantly poorer performance on the Bayley Psychomotor Developmental Index (PDI) than the children of women with higher free T_4 levels (N=198) (mean difference in scores 7.4; 95% CI 1.1 to 13.9), and were at an increased risk for a low score on psychomotor development (below 1 SD of the mean; RR 5.8; 95% CI 1.3 to 12.6). With linear regression, maternal free T_4 concentrations also demonstrated a significant positive correlation with infant scores on the PDI (R: 0.46, p=0.03). This study provided the first evidence that a subclinical maternal thyroid hormone deficiency could be influential in determining fetal neurodevelopment, specifically at a time before the fetus is able to act as its own supply of thyroid hormones. Extensive measures were taken to ensure that the population examined was healthy, so that any overt manifestation of disease could not be considered to be on the causal pathway to poor neurodevelopment. An interesting point to be noted though is that 46% of women with free T_4 levels <10th percentile at 12 weeks gestation had elevated thyroid peroxidase antibody (TPO-Ab) titres (>50 mU/L) at 32 weeks gestation, which is much higher than the 10% of normal adults that could be expected to have high antibody concentrations (56). Elevated TPO-Ab levels are commonly seen in a number of thyroid diseases, including nodular goiter, thyroid carcinoma, Graves' thyrotoxicosis, and chronic autoimmune thyroiditis (56), so this observation could possibly be an indication of an underlying condition not otherwise detected.

In a subsequent study, the effect of maternal hypothyroxinemia on infant neurodevelopment was examined longitudinally, with a follow-up period that lasted until the children reached two years of age (57). The subjects for this study were randomly selected from women who presented for antenatal care in and around Eindhoven, the Netherlands, between January 1997 and April 1998. Cases were defined as mothers that had free T_4 levels below the 10th percentile (12.4 pmol/L) and normal-range TSH levels at 12 weeks gestation (N=135), while controls had free T_4 levels in the 50th-90th percentile range (15.6 – 19.1 pmol/L) with normal TSH levels and were matched in a 1:1 ratio to cases on the basis of parity and gravidity (N=135). The infants were assessed for

neurological functioning at one and two years of age using the Bayley Scales of Infant Development, with analyses based on 63 cases and 62 controls after one year, and 57 cases and 58 controls after two. At both one and two years of age, the children of hypothyroxinemic women had significant, and clinically relevant, reductions in scores on indices of motor and mental development compared to the children of controls.

The effect of the interaction between weeks of gestation and changes in maternal thyroid hormone levels on neurodevelopment was also investigated using measures of maternal hormone levels at 12, 24, and 32 weeks gestation (57). In contrast to their earlier work in which maternal free T_4 levels at 32 weeks gestation had no influence on infant development (5), the authors found that the effect of hypothyroxinemia in early gestation could be mediated by changes over the course of the gestational period. Women who had low free T_4 at 12 weeks, but who experienced an increase throughout the latter portion of pregnancy gave birth to infants unmarked by the neurodevelopmental delays observed in those infants whose mothers did not experience this same phenomenon. Comparatively, the women with free T_4 levels that were below the 10^{th} percentile at 12 weeks and continued to drop throughout gestation gave birth to the infants with the poorest developmental profiles, while those with normal free T_4 at 12 weeks and a reduction throughout pregnancy were not affected. The latter two findings imply that the damage is sustained during the period in which the fetal thyroid gland is not yet functional, and therefore the fetus is entirely reliant on a maternal supply of thyroxine. They also suggest that once the fetus is able to produce its own supply of hormones, these stores are sufficient to maintain normal functioning and development, even if the mother is unable to sustain the expected contributions. However, the evidence suggesting that an increase in thyroxine levels can restore normal development is more difficult to make sense of, as it runs counter to the hypothesis that maternal thyroid hormones exert their most important effects during the first trimester. Assuming that this is not a chance finding, the possible conclusions that can be reached are that maternal hypothyroxinemia at 12 weeks is not as influential as has been suggested, with later availability of thyroid hormones from both the mother and the fetus being able to compensate for any prior deficiency, or that perhaps the increase in maternal free T_4 occurs to a great enough

degree between 12 and 16-20 weeks gestation, when the fetal thyroid gland becomes functional, that normal neurodevelopment can still be salvaged through the mothers' contribution. The therapeutic implications of these findings are that thyroxine supplementation could be beneficial in women suffering from thyroid hormone deficiencies. At this point though, there is no concrete evidence that thyroxine supplementation has beneficial effects on fetal and infant development.

In the most recent study on the effect of maternal hypothyroxinemia on neurodevelopment, infant performance was assessed at three weeks of age (58). Inclusion criteria were the same as in the study described above, but this study was set in the city of Veldhoven, rather than in Eindhoven. Maternal thyroid hormone assessments were also collected in the same manner, but neurodevelopment was assessed using the Neonatal Behavioral Assessment Scale (NBAS). In the neonates born to the cases (N=108), significantly lower scores ($p=0.04$) on the orientation index of the NBAS were observed than in the children of the cases (N=96). The orientation index is one of seven clusters that was analyzed and encompasses traits related to overall alertness and response to visual and auditory stimuli. Non-significant trends towards lower scores among the newborns of cases were also observed in the measurements of range of state (arousal) and regulation of state (ability to regulate state in response to increasing levels of stimulation) ($p<0.10$). While the NBAS is designed to generate scores on various separate indices, it is more importantly intended to gain an appreciation of how each of these scales interact with one another to provide a description of the overall development of the infant (59). A point of interest is then the significance of each of the scores in isolation. While hypothyroxinemic mothers may be at an increased risk of having an infant with a depressed score on the orientation index, a question to be raised is whether this will necessarily translate into a delayed developmental profile. Another issue to note is that while the authors suggest that these findings provide further support for their earlier work in which maternal hypothyroxinemia is associated with an increased risk of neurodevelopmental delay, the manifestations of this impairment do not seem to overlap. In both prior studies, the infants born to hypothyroxinemic mothers had an increased risk of a reduced score on the assessment of motor development (5,57), however this index

was not affected in the present study. A final note is that both maternal anxiety during pregnancy and gestational age at delivery were significant predictors of low scores on measures of orientation, bringing into question the significance of the contribution of maternal hypothyroxinemia.

Importantly, the studies conducted by this research group have provided evidence to challenge the previously held belief that subclinical alterations in thyroid hormone homeostasis were irrelevant in the context of fetal health, and have directed attention towards the possibility that since T_4 is the metabolic precursor of the physiologically active T_3, it is quite plausible that an inadequate supply of T_4 is indeed the root of neonatal problems attributed to maternal thyroid hormone deficiencies during pregnancy.

To date, there are only limited accounts of the non-neurological effects of maternal hypothyroxinemia on fetal development. Animal data collected using a rat model has suggested that maternal hypothyroxinemia induced by thyroidectomy results in reduced litter size and impaired fetal growth, with offspring exhibiting body weights 10% lower than those of controls (60). This effect however, was only observed during early gestation, before the beginnings of fetal thyroid function, after which fetal body weight normalized. A proposed mechanism to explain this effect is a reduction in the expression of the glucose transporter GLUT1 in the placenta and fetal brain. GLUT1 plays a critical role in the mediation of glucose transfer from the maternal to fetal systems, and a deficiency in this transporter translates into compromised glucose uptake (60). In hypothyroxinemic rats, GLUT1 expression was indeed reduced in the placenta as well as in the fetal brain, potentially contributing to the adverse birth outcomes observed (60). As with fetal growth though, this was only seen in early gestation. The ability of the fetus to recover from this type of insult calls into question the practical significance of this finding, but it is also possible that it is merely reflective of species differences between rodents and humans, indicating a less permanent effect of a thyroid hormone deficiency in the former. Finally, while the study describes its exposure in the rat dams as maternal hypothyroxinemia, TSH levels were not actually measured, and other descriptions of this rodent model have reported marked elevations in the measurements of

this hormone (61). These results therefore, may be more indicative of the effect of maternal hypothyroidism than hypothyroxinemia.

In humans, there is also some documentation that a perturbation of thyroid function is associated with IUGR, although the evidence is still limited and somewhat ambiguous. In a comparison of 26 fetal blood samples collected by cordocentesis from appropriately grown fetuses (gestational age 20-38 weeks) and 15 collected from fetuses compromised by IUGR (gestational age 24-35 weeks), Kilby *et al.* found that free T_4 and free T_3 levels were significantly lower in the latter group ($p<0.001$), with TSH levels remaining in the normal range, suggesting relative fetal hypothyroxinemia (7). These results were complemented by the finding that placental samples collected from pregnancies complicated by IUGR had increased expression of the thyroid hormone receptor variants TR $\alpha 1$, $\alpha 2$, and $\beta 1$, when compared to normal placentae ($p<0.01$ for each variant). Presumably the upregulation of thyroid hormone receptors in the placenta and the resultant fetal thyroid hormone deficiency stems from a maternal thyroid hormone deficiency, but data was not provided to relate the mother's thyroid economy to the resulting fetal condition (7). These findings are of interest due to the proposed role of the thyroid gland in the etiology of IUGR through its actions on the placenta. Stimulation of the production of 17β estradiol and epidermal growth factor in the placenta has been linked to the actions of T_3, suggesting a possible role of the hormone in placental growth and development (62), processes which are disrupted in IUGR pregnancies (63).

An interesting consideration in relation to these findings is the possibility of reverse causation. This would indicate that rather than an increased expression of thyroid hormone receptors acting as a marker of a factor that would induce IUGR (i.e. maternal thyroid hormone deficiency), IUGR would be the underlying cause of the receptor upregulation. The suggestion in this situation would be that feedback from the fetus, rather than from the mother, determines the compensatory mechanism required to make more thyroid hormones available.

In a study similar to the one described above, Chan *et al.* found that expression of monocarboxylate transporter 8 (MCT8), a highly specific thyroid hormone membrane transporter, was increased in placental samples associated with IUGR (8). When comparing normal placental samples to gestationally-matched IUGR samples, the observed increase in mRNA expression reached significance in placentae collected in the early third trimester (26-32 weeks gestation; $p<0.05$), but was no longer observed in the late-gestation group (37-38 weeks gestation). It is important to note that the data on IUGR was drawn from 10 cases, all of which were identified according to very strict criteria, resulting in the use of only severe cases of growth restriction. Third trimester placental samples in both the normal and IUGR groups were collected following Caesarean section, although the reasons were not specified in the IUGR group. The normal group underwent Caesarean sections for placenta previa, maternal tumors, or prelabour rupture of membranes with a breech presentation. Also, while the placental samples were approximately gestationally matched, the IUGR group had earlier deliveries in both the early- and late-gestation groups, with samples from 26-32 weeks being compared to 28-34 weeks, and those from 37-38 weeks being compared to term deliveries. These differences in sample collection and gestational age may confer additional discrepancies between the two groups beyond fetal growth restriction.

Placentae from normal pregnancies were collected from 6 weeks gestation to term, with measurements suggesting that MCT8 expression increases over the course of the pregnancy (8). Given that maternal thyroid hormone deficiencies are believed to have the greatest impact on fetal development during early gestation (5), it would be interesting to determine whether expression of MCT8 is altered in IUGR placentae in the first half of gestation. The finding that the upregulation of this transporter was observed in early but not late third trimester samples suggests that this protein may be more relevant earlier in gestation, possibly prior to the onset of fetal thyroid function.

The most direct evidence available on the relationship between maternal hypothyroxinemia and fetal growth restriction comes from a large study of 17,298 women published in 2007, in which the authors examined the effects of a maternal

thyroxine deficiency on perinatal outcomes and concluded that the condition is not associated with any change in the risk of adverse events (9). Casey *et al.* identified hypothyroxinemic women as those with TSH in the normal range and a free T_4 level at or below the 2.5% mark, resulting in concentrations ≤0.86 ng/dL (11.1 pmol/L). However, using this definition, only 1.3% (N=233) of their study subjects were considered to have hypothyroxinemia. The 10th percentile has been commonly used in the literature to describe hypothyroxinemia, including in the studies that have observed adverse effects on fetal and infant neurodevelopment (5,57,58). By limiting their definition of exposure to 1.3% of their population, Casey *et al.* may have reduced their ability to detect any association between hypothyroxinemia and the already rare outcomes of interest, including low Apgar score and fetal and neonatal mortality. In Casey's work, the cohort was derived from women who were screened for rubella during the first half of pregnancy, with tests ranging from 6-20 weeks gestation. The 11.1 pmol/L value that was used in their definition of exposure was calculated based on all of these women and was applied uniformly. However, free T_4 concentrations do not remain constant throughout pregnancy. Following an initial transient increase in response to hCG secretion, free T_4 concentrations drop over the course of the remainder of the pregnancy (35). Although the data is not shown, Casey reports that gestational age-specific free T_4 cut-offs were also tested in analyses and the results did not differ from those using an averaged 2.5% measurement.

To measure fetal growth restriction, birth weight was selected as an outcome, measured as ≤1,000g and ≤2,500g, without consideration of gestational age (9). While birth weight is easily obtained, a more accurate depiction of fetal growth may have been gained from the use of small for gestational age or birth weight z-scores. Another outcome that was assessed was the five minute Apgar score, using a cut-off point of a score <3. A five minute Apgar score <3 is highly correlated with neonatal mortality, being associated with a RR of 1460 (95% CI 835 to 2555) (26). This is a very rare outcome though, and indeed, no cases were reported among women with maternal hypothyroxinemia. Additionally, neonatal mortality was among the outcomes measured. However, no cases were observed in the study group, therefore the measure of a low Apgar score may have

been more informative if it had included more observed data. This may have been possible through the use of an Apgar score <7 as clinically, scores of 7-10 are considered to be good to excellent, while those <7 are considered to have a poorer prognosis (26). Finally, while Casey *et al.* adjusted their findings to account for the influence of maternal age, race, parity, and weight, there are several more known risk factors for adverse perinatal outcomes, notably maternal smoking. By accounting for these additional factors, it would be possible to provide a more realistic portrayal of the risk incurred by maternal hypothyroxinemia.

The findings of these three studies imply that maternal thyroid hormone deficiency may play a role in stimulating compensatory mechanisms at the level of the placenta to increase fetal thyroid hormone availability, and that fetal hypothyroxinemia may be associated with growth restriction, but the evidence is limited as to whether maternal hypothyroxinemia plays a significant role in determining the course of fetal growth.

The most common cause of maternal hypothyroxinemia is believed to be an iodine deficiency, as this occurs more frequently than primary thyroid dysfunction or thyroid autoimmunity, making it an easily preventable condition (36). While Western societies have generally been considered to be iodine-sufficient, recent trends have shown that the proportion of the population in which this is true is declining, especially among pregnant women. It is recognized that pregnant women have an increased demand for iodine, and the recommended daily intake is 250μg (200-300μg), compared to 150μg for other adults (64). According to data from the National Health and Nutrition Examination Survey (NHANES) conducted in 2001-2002, 7.3% of pregnant women were iodine-deficient (urinary iodine <50μg/L), compared to 6.9% between 1988 and 1994 (64). Previous results collected from NHANES have been shown to be comparable to Canadian data from the same time period (65), making these levels of iodine-deficient women a concern in this population as well. Currently, it is unknown the proportion of Albertan women who are iodine deficient, but the default assumption in clinical practice is that of iodine sufficiency.

1.1.4.4.3. Hyperthyroidism

In contrast to the conditions described above in which a deficiency in thyroid hormones leads to adverse effects, hyperthyroidism describes a condition in which serum TSH levels are reduced, nearly always below 0.1 mU/L, and free T_3 and T_4 concentrations are elevated (66). It is quite rare in pregnancy, with an estimated prevalence of 0.1-0.2% (39,66). The majority of this proportion is made up of cases of gestational transient thyrotoxicosis (GTT), a nonautoimmune condition resulting from the stimulation of the thyroid gland by hCG (66). While GTT may be associated with maternal symptoms such as weight loss, tachycardia, and fatigue, it has not been linked to adverse pregnancy outcomes (66). The other main cause of hyperthyroidism in pregnancy is Graves' disease. Unlike GTT, Graves' disease is autoimmune in origin and its consequences are typically more severe. Common symptoms include fatigue, palpitations, anxiety, heat intolerance, diaphoresis, and weight loss, all of which, with the exception of weight loss, are associated with a normal pregnancy, potentially making the condition difficult to recognize (66). If left unmanaged, Graves' disease can be associated with preeclampsia, fetal malformations, premature delivery, and low birth weight; however, if treated, the prognosis for both the mother and fetus is good (66).

While this section is not by any means a complete review of all of the thyroid diseases that may occur during pregnancy, its purpose is to act as a brief summary of some of the major conditions, with an emphasis on those in which a deficiency in thyroid hormones is the main clinical feature, due to evidence that this insufficiency may play a role in the etiology of fetal growth restriction.

1.2. Hypothesis and research objectives

With evidence suggesting that hypothyroxinemia can alter the course of fetal development, it was hypothesized that a deficiency in maternal free T_4 would translate into adverse pregnancy outcomes. This thesis is designed to determine whether maternal hypothyroxinemia at 15-16 weeks gestation increases the risk of fetal growth restriction and/or early neonatal morbidity.

Table 1.1. Apgar score.

Sign	Score		
	0	1	2
Color	Blue or pale	Acrocyanotic	Completely pink
Heart Rate	Absent	<100 beats/min	>100 beats/min
Reflex/Irritability	No response	Grimace	Cry or active withdrawal
Muscle Tone	Limp	Some flexion	Active motion
Respiration	Absent	Weak cry: Hypoventilation	Good, crying

American Academy of Pediatrics, Committee on Fetus and Newborn, American College of Obstetricians and Gynecologists and Committee on Obstetric Practice. Pediatrics. 2006;117:1444-1447.

Table 1.2. Factors associated with, and influential in determining Apgar score.

Low Apgar without fetal acidosis or hypoxia	Normal Apgar with acidosis
Immaturity	Maternal acidosis
Analgesics, narcotics, sedatives	High fetal catecholamine levels
Magnesium sulfate	
Acute cerebral trauma	
Precipitous delivery	
Congenital myopathy	
Congenital neuropathy	
Spinal cord trauma	
Central nervous system anomaly	
Lung anomaly (diaphragmatic hernia)	
Airway obstruction (choanal atresia)	
Congenital pneumonia and sepsis	
Previous episodes of fetal asphyxia (recovered)	
Hemorrhage-hypovolemia	

Adapted from Kliegman RM, Behrman RE, Jenson HB and Stanton BF eds. Nelson Textbook of Pediatrics, 18th edition. United States of America: Saunders Elsevier; 2007. Accessed June 4, 2008 at <http://www.mdconsult.com.login.ezproxy.library.ualberta.ca/das/book/body/96441976-3/712433972/1608/273.html#4-u1.0-B978-1-4160-2450-7..50096-7--cesec22_2454>.

Table 1.3. Physiologic effects of thyroid hormones.

Target tissue	*Effect*	*Mechanism*
Heart	Chronotropic Inotropic	Increase number and affinity of β-adrenergic receptors Enhance responses to circulating catecholamines Increase proportion of α myosin heavy chain (with higher ATPase activity)
Adipose tissue	Catabolic	Stimulate lipolysis
Muscle	Catabolic	Increase protein breakdown
Bone	Developmental	Promote normal growth and skeletal development
Nervous system	Developmental	Promote normal brain development
Gut	Metabolic	Increase rate of carbohydrate absorption
Lipoprotein	Metabolic	Stimulate formation of LDL receptors
Other	Calorigenic	Stimulate oxygen consumption by metabolically active tissues (exceptions: testes, uterus, lymph nodes, spleen, anterior pituitary) Increase metabolic rate

Ganong WF, ed. Review of Medical Physiology. 20th edition. United States of America: McGraw-Hill; 2001:307-321.

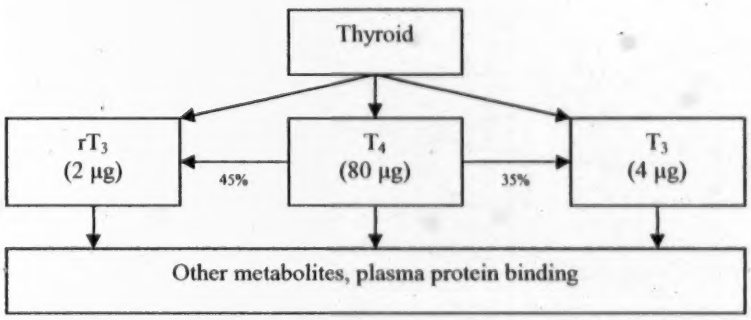

Figure 1.1. Thyroxine metabolism.

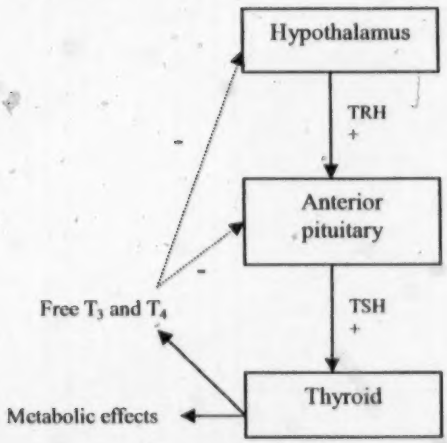

Figure 1.2. Hypothalamic-pituitary-thyroid axis. The solid arrows indicate stimulatory effects and the dashed arrows indicate inhibitory effects.

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Chapter 2

Maternal Hypothyroxinemia, Fetal Growth Restriction, and Early Neonatal Morbidity

2.1. Introduction

Normal fetal development is programmed to a large extent by the availability of thyroid hormones, and sufficient amounts of triiodothyronine (T_3) and thyroxine (T_4) are crucial in the promotion of proper cardiac, pulmonary, and central nervous system functioning (1). Despite this requirement, the fetal thyroid gland does not fully develop until mid-gestation, necessitating a dependence on a maternal supply of thyroid hormones prior to this point (2). Thyroid hormone deficiencies are known to be associated with a perturbation of normal developmental processes, and maternal hypothyroidism, in which thyroid-stimulating hormone (TSH) levels are reflexively elevated above the 98th percentile (3), implying a deficiency in T_3 and T_4, has long been associated with adverse pregnancy outcomes, including fetal growth restriction, motor rigidity, auditory deficits, and mental retardation (4,5). Though data is available on the consequences of hypothyroidism, less is known about the effects of hypothyroxinemia, a related thyroid hormone deficiency.

Hypothyroxinemia occurs when free thyroxine levels are below the 10th percentile for a population, without a corresponding increase in TSH (6). Although this has previously been considered a subclinical condition, research is beginning to emerge that challenges this assumption. In a series of studies conducted by Haddow *et al.* (7) and by Pop *et al.* (8-11), thyroid deficiencies limited to the mother, including maternal hypothyroxinemia, have been linked to poorer infant psychomotor development, impaired neuropsychological development, and breech presentation. Stemming from the evidence presented in these publications, the impact of hypothyroxinemia on various perinatal outcomes has also been considered. A recent American study of 17,298 women examined the association between isolated maternal hypothyroxinemia (defined in this study as free T_4 <0.86 dL and normal-range TSH before 20 weeks gestation) and adverse neonatal outcomes, including low birth weight, Apgar score <3, and fetal or neonatal

death, although no significant effects were observed (12). Reduced concentrations of circulating free T_4 and free T_3 with normal TSH levels have been documented in blood samples drawn from growth-restricted fetuses (13), and in placental samples collected from intrauterine growth restricted (IUGR) births, increased expression of thyroid hormone variants TR α1, α2, and β1, as well as a thyroid hormone membrane transporter, monocarboxylate transporter 8 (MCT8), has been found (13,14). These results suggest that thyroid hormone deficiencies are sufficiently important to the fetus to warrant the triggering of compensatory mechanisms.

Hypothyroidism and hypothyroxinemia are related by a shared free T_4 deficiency, differing only in the compensatory actions of TSH. The present study was designed to assess whether maternal hypothyroxinemia is associated with fetal growth restriction and early neonatal morbidity, and consequently, whether an inadequacy of free T_4 underlies the adverse effects associated with thyroid hormone deficiencies, regardless of TSH concentrations.

2.2. Methods

2.2.1. Study population

The study was conducted in the Capital Health Region, an administrative unit within the universal healthcare infrastructure, in and around Edmonton, Alberta, Canada. Women who were pregnant in 2005-2006 and who elected to undergo a second trimester prenatal screen for Down syndrome, open spina bifida, and trisomy 18 made up the study population. Screens were collected between December 15, 2005 and June 22, 2006. Only women receiving the screen at 15-16 weeks gestation, and those who had been referred by a physician who had made at least eight similar recommendations over the course of the study period were included, the latter criteria intended to minimize the risk of over-representation of high-risk pregnancies. Further criteria that were used to determine the inclusion of study subjects were maternal age ($\geq$18 years) and live singleton deliveries $\geq$22 weeks of gestation. Additionally, any women who gave birth to infants with malformations, as determined by data collected at the time of birth, were

excluded from the analysis. The study protocol received ethical approval from the Health Research Ethics Board at the University of Alberta.

2.2.2. Pregnancy outcome assessment and perinatal risk factors

The outcomes of interest in this study were early neonatal morbidity, measured by five minute Apgar score, and fetal growth restriction, measured as small for gestational age (SGA: birth weight $<10^{th}$ percentile for gestational age and gender) and z-score for birth weight (15). Outcome data was obtained from the Delivery Record that is collected at the time of birth in all hospital and midwife-attended deliveries, encompassing >99% of all provincial births (Appendix I). Information from the Delivery Record is stored electronically by the Alberta Perinatal Health Program (APHP) and was obtained from this organization (www.aphp.ca).

The maternal characteristics collected from APHP included age, weight, height, substance use (including cigarettes, alcohol, and illicit drugs), obstetric history (gravida; previous SGA births; poor weight gain; pregnancy type, i.e. singleton or multiple), and medical history (diabetes – pre-existing and gestational; cardiovascular conditions – heart disease, hypertension, pregnancy-induced hypertension, and proteinuria; renal disease; anemia). Women with both pregnancy-induced hypertension and proteinuria were classified as having preeclampsia. Infant variables included gender, birth weight, gestational age, fetal anomaly (suspected and observed), and stillbirth. Maternal race and gestational age at the time of serum collection were also available from data collected as a part of the prenatal screen.

2.2.3. Thyroid hormone assessment

Serum samples were stored at -20°C until the time of analysis for thyroid hormones. Thyroid function was determined based on maternal serum TSH and free T_4 concentrations measured by an automated analyzer (Bayer Advia Centaur) employing direct chemiluminescent technology. Concentrations of free T_4 were measured using a competitive immunoassay. In this analysis free T_4 present in the serum sample competes with acridinium ester-labeled T_4 in the Lite Reagent for binding sites on a constant

amount of polyclonal rabbit anti-T_4 antibodies, which are covalently bound to paramagnetic particles present in the solid phase. An inverse relationship exists between the amount of light units emitted and the amount of free T_4 in the patient's sample.

Each of the serum samples collected was screened for TSH using a two-site sandwich immunoassay. Briefly, a fixed amount of two antibodies was used: a monoclonal mouse anti-TSH antibody labeled with acridium ester introduced via the Lite reagent, and a solid phase polyclonal sheep anti-TSH antibody that is covalently coupled to paramagnetic particles. The amount of TSH in the patient sample is directly related to the amount of relative light units detected by the system.

The inter-assay coefficients of variation for the measurements of TSH were 10%, 2.7%, and 2.1% at 0.06, 10.8, and 25.2 mU/L, respectively. The corresponding values for the measurements of free T_4 were 3.9%, 3.0%, and 3.6% at 4.8, 18.4, and 60.5 pmol/L, respectively (16).

From these analyses, each of the women was classified into one of four groups: euthyroid (TSH 0.15-4.0 mU/L; free T_4 >8.5 pmol/L), hypothyroxinemic (TSH 0.15-4.0 mU/L; free T_4 ≤8.5 pmol/L), hypothyroid (TSH >4.0 mU/L), or hyperthyroid (TSH <0.2 mU/L). These end-points were based on the reference ranges used by the laboratory staff at Capital Health in routine thyroid function measurement.

2.2.4. Statistical analysis

All statistical analyses were conducted using STATA version 8.0 (StataCorp, College Station, TX). Differences in continuous variables were assessed using the *t*-test (two-tailed), and those in categorical variables were analyzed with Fisher's exact test. Risk ratios (RR and associated 95% confidence intervals (CI)) were estimated for SGA and Apgar score (<7 at five minutes) using a modified Poisson regression procedure (17). Linear regression was used to evaluate birth weight z-score. All potential confounders were included in the regression models, as well as interpretable interaction terms that

reached statistical significance. All outcomes were tested for associations with hypothyroxinemia and with free T_4 concentrations. Statistical significance was set at 5%.

2.3. Results

2.3.1. Thyroid hormone measurements

The serum samples of 974 women who had the prenatal screen during the study period were analyzed (Table A2.1, Figures A2.1 – A2.7). Of these 974, the screens of 902 were successfully linked to their corresponding delivery records by APHP. A further 23 were excluded: 7 duplicate samples from the same woman, 5 twins, 8 infants with observed anomalies, 1 stillbirth, 1 implausible birth weight for gestational age (standard deviation (SD) 14.6 - confirmed data entry error), and 1 infant born earlier than 22 weeks of gestation. In the case of a repeated measurement in a single woman, her first record was retained. Ultimately, 879 women were included in the cohort (Figure 2.1). Measurements of TSH ranged from 0.01 to 9.31mU/L in the entire cohort, with a mean value of 1.16 mU/L (SD 0.94). Free T_4 levels spanned 4.2 to 27.1 pmol/L, with a mean of 11.7 pmol/L (SD 2.6) and geometric mean of 11.0 pmol/L (95% CI 5.8 to 19.3 pmol/L). The women of the cohort were stratified according to four maternal hormone classifications of interest: euthyroid (N=756), hypothyroxinemic (N=89), hypothyroid (N=15), and hyperthyroid (N=19). Details of thyroid hormone levels for both the cohort as a whole, and as stratified by thyroid hormone status, are provided in Table 2.1. The two hormones were only weakly correlated, with a correlation coefficient (r) of 0.19 (Figure 2.2).

Serum levels of TSH were not significantly changed over the two week collection period (15-16 weeks gestation), with a reduction of 0.0042 ln-units per day (95% CI -0.021 to 0.013). Free T_4 levels, however, were associated with a slight decrease of 0.068 pmol/L per day (95% CI -0.12 to -0.013) (Figure 2.3). The repeated measurements made in the same woman support this trend. In five of the seven women who had duplicate screens, serum free T_4 levels were reduced at the time of the second test when compared to the first (Figure 2.4). However, when tested as a potential effect modifier in the regression

models, the interaction between free T_4 concentrations and the gestational age at the time of the test was not significant.

2.3.2. Comparison of the study cohort to the Alberta population

Details on the demographics and pregnancies of the women for whom data linkage was complete, those who were not linked, and the final study subjects are provided in Table 2.2. The women who were not linked at APHP were significantly younger, with a mean age of 28.7 years, compared to 30.6 years in the linked group ($p<0.01$). The proportion of women under the age of 25 was also significantly higher (<20: $p<0.01$; 20-24: $p=0.05$). However, besides maternal age, the linked and not linked groups showed no appreciable differences in maternal race, thyroid hormone status, gestational age at time of test, or month of prenatal screen. Similarly, there were no significant differences observed between the linked group and the final study subjects.

The study subjects were also compared to all women in Northern Alberta receiving the prenatal screen between December 2005 and February 2008 (N=15,006) on the same parameters as described above, with the exception of maternal thyroid hormone status as only the samples collected from study subjects were analyzed (Table 2.2). The mean maternal age in the study group was significantly higher than that of the reference group (30.6 compared to 29.8; $p<0.01$), with a higher proportion of women in the age group 30-34 ($p<0.01$) and a lower proportion in the age groups <20 and 20-24 ($p=0.01$ and <0.01, respectively). These findings are not surprising since the greater population included women 14 years of age and older, shifting the mean towards a younger age, while also increasing the observed frequency of women <20. In most cases, maternal race was comparable between the two populations. However, more women of Asian, Filipino, and Oriental descent were observed in the study population, while fewer First Nations women were represented. The gestational age at the time of the screen was significantly lower in the study population, with a mean of 112.6 days compared to 116.6 days. This was also not unexpected as we were limiting our data collection to women who had undertaken the screen at 15-16 weeks gestation. Finally, the month of the prenatal screen differed between the two populations. In the study group, serum samples were only collected

between December 2005 and June 2006, explaining the significant differences in proportions when compared to the year-round collection encompassed by the reference population. However, when the comparison was limited to the months of January to June, as well as December, in both populations, the two groups were still significantly different in every month but February. The trend that was observed was that in the winter months (December to March), the larger population had a higher proportion of women screened than were screened in the study group, but this was reversed in the spring (April to June), when the larger population had a lower proportion of women screened. The practical significance of these results is uncertain.

As of February 2007, the second trimester prenatal screen has been a standard of care, but at the time of sample collection, it was still an elective procedure. Typically the screen was recommended due to a high risk pregnancy, especially for older mothers, or for those with previous complications in pregnancies or previous babies with congenital anomalies. However, some physicians offered it routinely, and some women requested the screen due to personal preference (18). A requirement for inclusion in the study was that the woman had to have been referred for the screen by a physician making at least eight similar recommendations over the study period, with the intention of minimizing the chance that high-risk pregnancies would be over-represented. Therefore, it can be hoped that the study subjects were more representative of the average population in their respective age strata.

A caveat to be noted is that only current thyroid hormone concentrations could be determined. The possibility that there were women in the cohort with preexisting hypothyroidism who were adequately treated cannot be discounted. In name, this could introduce exposure misclassification, although for all intents and purposes, these women would be equivalent to the physiologically euthyroid women with whom they would be grouped. As there were 15 hypothyroid women in our cohort, it is evident that there were untreated or inadequately treated study subjects in our population, negating concern that we were unable to collect data on this group.

The study subjects were in all likelihood healthier than could be expected in the rest of the province. The study subjects were selected from a group of women that had taken a prenatal screen during their second trimester of pregnancy, indicating that they all had access to a physician and/or prenatal care, known preventive factors in the development of adverse pregnancy outcomes. In a comparison of the study cohort to province-wide data published in 2006 (19), it was found that the study subjects had lower rates of preterm delivery, self-reported smoking and alcohol consumption than Albertan women as a whole, supporting the assumption that the cohort was a healthier group. The mean maternal age at delivery was very similar between the data from the cohort and from the Alberta figures, but a higher proportion of women aged ≥35 was represented in the study population (19). Additionally, stillbirths, fetal anomalies, and multiple deliveries were excluded, limiting the study population to lower risk pregnancies. A comparison of these two populations is provided in Table 2.3.

2.3.3. Simple stratified analyses

The mean birth weight amongst the infants born to the women included in the study was 3378 g, the mean z-score was 0.076, 61 were classified as SGA (6.9%), and 9 had a five minute Apgar score <7 (1.0%). Simple stratified analyses suggested that younger women (18-24), those of smaller stature (weight and/or height), and those who had previously given birth to an SGA baby were at an increased risk of adverse pregnancy outcomes (Tables 2.4, A2.2).

Among infant variables, gender was fairly evenly matched, there were two infants with suspected anomalies, neither of which were observed to have an anomaly at birth, and the rate of preterm birth was 7.5%, a number lower than the provincial average of 9.1% in 2005 (20). Maternal thyroid hormone status divided the study subjects into four groups. Based on our definition of hypothyroxinemia encompassing women with serum free T_4 levels at or below the 10^{th} percentile, our observed proportion of these women (10.1%) is appropriate. Hypothyroidism is observed in 1-2% of the population during pregnancy (5), a frequency which was mirrored in our population. The proportion of hyperthyroid women in our population is slightly problematic however. In the general population,

hyperthyroidism in pregnancy is rare, being observed in approximately 0.1-0.2% of women. Our observed frequency of 2.2% is notably higher, which is likely due to a selection bias, as women with a higher risk of complications in pregnancy, or overt clinical symptoms, would likely have a higher probability of being screened.

Maternal race was examined using Caucasian women as the reference group. In all other categories, the mean birth weight was lower, significantly among Asian, First Nation, and Oriental women. When adjusting for gender and gestational age, this relationship remained significant among Asian women, with an RR for SGA of 2.67 (95% CI 1.47 to 4.86). The trend was reversed among First Nations women: the mean z-score for birth weight was 0.26 (SD 0.51), compared to 0.17 (SD 0.94) among Caucasian women. First Nations women were also more likely to have infants with Apgar scores <7, with a RR of 9.06 (95% CI 1.14 to 72.29).

Past obstetric history in our population was captured through the use of variables for gravida and previous SGA delivery. An increase in gravida demonstrated a trend towards a positive relationship with birth weight, corresponding to a reduced risk of the delivery of an SGA infant, as well as a reduced risk for an Apgar score <7. Conversely, previous SGA delivery was highly associated with a current SGA delivery, as well as an increased risk of a low Apgar score.

A number of anthropometric descriptors were available to us. Among women with a pre-pregnancy weight >91 kg, the risk of SGA was decreased, while women with a pre-pregnancy weight <45 kg, as well as those with a height <152 cm, were subject to an increased risk. Poor weight gain during pregnancy was also associated with an elevated risk of the delivery of a small infant. None of the women included in these categories gave birth to an infant with an Apgar score <7.

The only significant association that was found between maternal disease and fetal growth restriction was for the effect of preeclampsia (RR for SGA 3.69; 95% CI 1.08 to 12.58). Trends, however, suggest that women with diabetes are at a lower risk of having

an SGA infant, while women with hypertension may be at a slightly elevated risk. Again, none of these women had an infant with a low Apgar score.

Maternal smoking was reported in 11% of our population. Alcohol consumption and drug dependence were reported much less frequently, but still occurred. Smokers showed a trend towards an increased risk for delivering infants with SGA and a low Apgar score, which is consistent with the available literature (21,22). There were no SGA infants born to women who consumed alcohol or were drug dependent, nor were there any with low Apgar scores in the drug-dependent group. However, among women who consumed alcohol during their pregnancy, the risk of an Apgar score <7 was highly elevated, with a RR of 21.85 (95% CI 3.32 to 143.90).

2.3.4. Multivariate analyses

All of the available covariates were included in the regression models, and after this adjustment maternal hypothyroxinemia was not observed to have an association with SGA, birth weight z-score, or Apgar score <7. Using euthyroid women as the reference group, the RR for hypothyroxinemic women giving birth to an SGA infant was 0.38 (95% CI 0.11 to 1.33) and the mean difference in z-score was 0.038 (95% CI -0.17 to 0.24). None of these women gave birth to an infant with an Apgar score below 7 (Table 2.5). Free T_4 levels were also substituted for hypothyroxinemia in each of the regression models to determine whether a trend existed with the hormone concentrations and any of the selected outcomes, but no relationship was observed (Figure A2.8, Table A2.3). An increase of one picomole per liter free T_4 was associated with RRs of 1.29 (95% CI 0.69 to 2.43) for SGA and 1.11 (95% CI 0.21 to 5.82) for Apgar <7, as well as a slope of -0.11 (95% CI -0.35 to 0.13) for z-score (Table 2.6).

Consistently in the adjusted models, fetal growth restriction was significantly associated with poor maternal weight gain over the course of the pregnancy and a previous SGA delivery. Asian women were also at an increased risk of having an SGA baby, as well as one with a reduced z-score. Contrary to expectations, maternal smoking was not found to significantly increase the risk of SGA, or to significantly reduce z-score. Factors

associated with an increased risk of Apgar <7 included previous SGA delivery and any maternal alcohol consumption during pregnancy. Protective factors against fetal growth restriction included increasing maternal age and increasing gravida. Additionally, a longer gestational period reduced the risk of observing a low Apgar score (Table 2.6). The relationship between birth weight and free T_4 concentrations was examined when stratified by maternal smoking status with no observed effect (Figures 2.4, A2.9 – A2.10, Table A2.4). Significant interactions were noted between free T_4 levels and maternal age in the models for z-score and Apgar, however the associations that were implied were unable to be interpreted, and were therefore not included in the final models. There was no other evidence of effect modification (Tables 2.6, A2.5 – A2.8).

Based on the findings of a recent publication that suggested that the accuracy of the assessment of fetal growth differs between preterm and full-term infants due to the use of references developed from the weights of live births only (23), each of the regressions was run again, stratified according to gestational age (<37 weeks vs. ≥37 weeks). As shown in Tables 2.5 and 2.6, the birth weight z-scores among full-term infants do not differ from those of the study population as a whole.

Hypothyroidism was observed to be associated with a slight increase in mean z-score (0.48), but there were no instances of SGA or Apgar <7. Hyperthyroid women also demonstrated a slightly elevated mean z-score (0.13), and 1 gave birth to an SGA infant (5.3%). There were no infants born to hyperthyroid women with a low Apgar score (Table 2.5).

2.4. Discussion

This study was conducted to determine whether maternal hypothyroxinemia is associated with an adverse effect on fetal growth and early neonatal morbidity. While it was hypothesized that a reduction in maternal free T_4 levels would be detrimental to the development of the fetus, the results do not provide evidence to support this relationship.

The cohort selected was drawn from a pool of women who had undergone a second trimester prenatal screen at 15-16 weeks of gestation. The utility of this screen is limited by the fact that it is an optional test, typically offered to women suspected to be high-risk pregnancies due to increased age or a history of complications in pregnancy. However, the major benefit of drawing from this group of women was the timeframe it provided. As the fetal thyroid gland does not become functional until 18-20 weeks of gestation, it was advantageous that the maternal serum samples were analyzed for hormone concentrations at a point prior to this milestone. In this way, it could be determined whether a free T_4 deficit attributable solely to maternal thyroid function would have a detrimental effect on the development of the fetus, an issue of potential therapeutic importance. Thyroxine supplementation is common in the treatment of hypothyroidism, and has therefore been supposed to be a plausible intervention in the management of hypothyroxinemia. However, as it is not yet known if maternal hypothyroxinemia can be causally linked to adverse outcomes, the benefits of treatment remain uncertain.

As expected, the women whose delivery records were unable to be linked to the data from their prenatal screens by APHP were significantly younger than the women whose records were linked. The inclusion criteria contained a requirement for women involved in the study to be $\geq$18 years of age, explaining the higher proportion of women younger than 20 in the group that was not linked. Women between 20 and 24 years of age were also more highly represented in the unlinked group than in the linked group, possibly due to an increased likelihood of termination.

The observed rates of SGA were slightly lower than expected, but there are a number of potential explanations for these findings. The definition used for SGA was taken from the work of Kramer *et al.* (15) who published tenth percentile cut-offs for a large population of Canadian births between 1994 and 1996. However, it has been observed that the birth weight of Canadian newborns has been steadily increasing over time (15). Therefore, the 6.9% rate of SGA is likely partially explained by this trend. It is also plausible that the study population was in better health and had better access to medical care than could be expected from the general population. Since serum samples were

taken from a prenatal screen, it is evident that the entire cohort had access to a physician and/or prenatal care, factors that are known to reduce the likelihood of harm to the fetus (24). The suggestion that this specific population is healthier than average is also supported by the fact that 11% of women reported smoking at any time during pregnancy, a number well below the reported rate of 25.7% province-wide (19).

Previous SGA delivery was found to be a significant risk factor for all three outcomes. However, the rate of smoking among these women was 50%, a number more than four times greater than that reported in the overall cohort. Of the women who had a previous SGA delivery, all were euthyroid, further failing to provide support for the hypothesis that these adverse birth outcomes are the result of thyroid hormone deficiencies.

The observed trends relating to the impact of maternal smoking on fetal growth restriction were reflective of the current literature. Although not significant, the risk of SGA was increased approximately two-fold among smokers, which is similar to other published estimates (21,22). The mean z-score for birth weight among smokers was reduced, as was expected, and this was apparent to a slightly higher degree among women who gave birth to full-term infants. It is likely, therefore, that in the full study population, the slightly lower effect estimate of effect encompassed some noise related to the induction of preterm delivery also associated with maternal smoking. It was anticipated that alcohol consumption and drug use would also be associated with fetal growth restriction, but possibly due to underreporting and to the fact that the information collected on illicit substances related to drug dependence and not necessarily drug use, this association was not found.

The findings of this study in relation to the effects of thyroid hormones are in agreement with those of Casey *et al.* (12), with the added strengths of adjustment for factors known to be influential in fetal growth (gestational age and maternal smoking status), and the use of a 10^{th} percentile cut-off for free T_4 levels in the definition of hypothyroxinemia. The allowance for the effect of gestational age on fetal growth is necessary in the distinction between an infant that is born small due to prematurity and one that has

suffered from a pathologic condition, while maternal smoking is strongly associated with adverse effects during pregnancy, particularly with a reduction in birth weight (21). The use of a higher cut-off point for free thyroxine levels, at 10% rather than at 2.5%, allowed for increased power to detect relevant outcomes, as well as ensured consistency with a large proportion of published work, allowing comparisons to be made between studies ((8-10)). Although a higher percentile was used, the 10^{th} percentile in the current study corresponded to a free T_4 concentration of 8.5 pmol/L, compared to 11.1 pmol/L (0.86 ng/dL; 95% CI 10.3 to 24.5 pmol/L) at the 2.5 percentile in the work of Casey *et al.* The serum samples were treated in a similar manner in each study, but differences in measurements may have arisen due to the use of a different instrument for the analysis (Bayer Advia Centaur compared to Immulite 2000 Analyzer), as well as the fact that the assays for free T_4 measurements are recognized to be problematic (25). Finally, while the five minute Apgar score was used as a measure of early neonatal morbidity in both studies, the present use of a score <7 rather than <3 was chosen due to its widespread use as a clinically relevant indicator of a poor to intermediate condition (26), as well as its capacity to increase power. Despite differences in study design, the major conclusions that a deficiency in maternal free T_4 is not significantly associated with a reduced birth weight or Apgar score are shared.

The seemingly contradictory finding to the literature that birth weight z-scores were elevated among hypothyroid women raised concerns that the study population may not be representative of typical hypothyroid patients. To determine whether our definition and classification of hypothyroidism was similar to that of other populations, free T_4 levels from the hypothyroid women in our cohort were compared to the data collected by Haddow *et al.* (7) who used a comparable screening procedure in the selection of study subjects. Their reported geometric mean value for free T_4 among hypothyroid women was 9.1 pmol/L (95% CI 7.4 to 11.2 pmol/L), which is only slightly lower than our value of 11.0 pmol/L (95% CI 5.8 to 19.3 pmol/L). In fact, overlap was observed in the distribution of free T_4 concentrations between the two populations, increasing confidence in our measurements.

One limitation of the present study was the inability to directly measure fetal growth restriction. SGA is a frequently used and easily obtained birth outcome measurement, but it is not without its drawbacks. SGA is commonly assumed to mirror IUGR and is therefore typically used as a proxy, but the two definitions are distinct and it is possible for an infant to be SGA without having suffered from growth restriction, and vice versa.

Finally, information was gathered on as many known risk factors of fetal growth restriction as possible, but there were certain elements that were unable to be captured. Data on socioeconomic status and intervals between pregnancies were not available, although it is known that both of these factors can contribute to adverse birth outcomes.

2.5. Conclusion

With non-significant estimates of risk for small for gestational age, birth weight z-score, and five minute Apgar score <7 among hypothyroxinemic women, it appears unlikely that this condition is detrimental to fetal development. From the data collected, a plausible conclusion is that hypothyroxinemia, being a subclinical physiological condition, does not have an appreciable effect on fetal growth restriction or early neonatal morbidity.

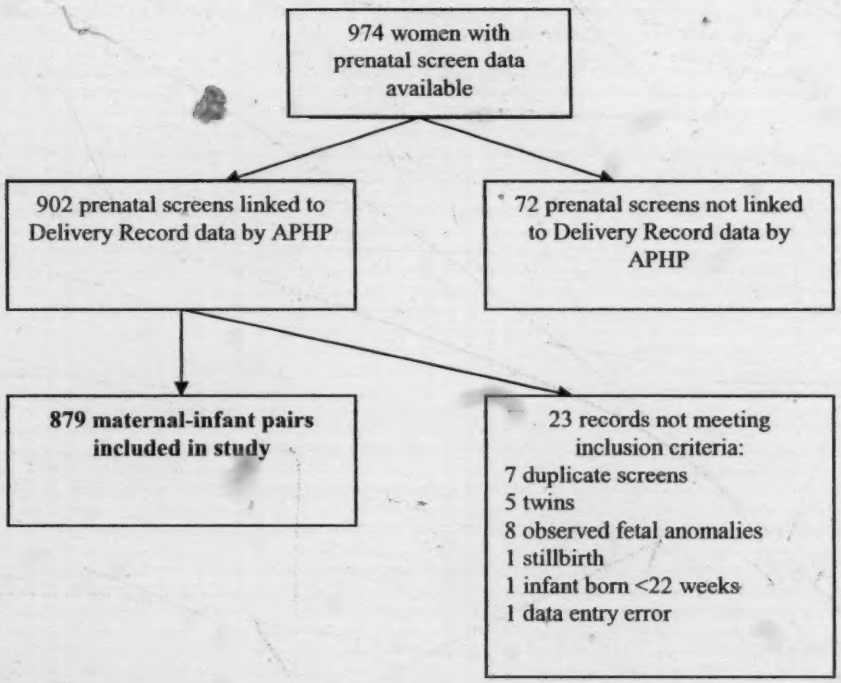
974 women with prenatal screen data available
902 prenatal screens linked to Delivery Record data by APHP
72 prenatal screens not linked to Delivery Record data by APHP
879 maternal-infant pairs included in study
23 records not meeting inclusion criteria:
7 duplicate screens
5 twins
8 observed fetal anomalies
1 stillbirth
1 infant born <22 weeks
1 data entry error

Figure 2.1. Study subject inclusion.

Table 2.1. Measurements of maternal thyroid function among study subjects*.

Study group (N)	*TSH (mU/L)*		*Free T_4 (pmol/L)*	
	Mean ±SD	*Median (IQR)*	*Mean ±SD*	*Median (IQR)*
All subjects (879)	1.16 ±0.94	0.94 (0.56-1.47)	11.7 ±2.6	11.6 (9.9-13.4)
Euthyroid (756)	1.15 ±0.71	1.01 (0.61-1.50)	12.2 ±2.2	11.9 (10.5-13.6)
Hypothyroxinemic (89)	0.68 ±0.36	0.61 (0.41-0.86)	7.6 ±0.8	7.8 (7.0-8.3)
Hypothyroid (15)	5.75 ±1.60	5.16 (4.46-6.79)	11.0 ±3.2	10.3 (8.4-14.6)
Hyperthyroid (19)	0.07 ±0.04	0.06 (0.04-0.11)	12.5 ±4.9	11.7 (9.2-15.3)

*IQR = interquartile range.

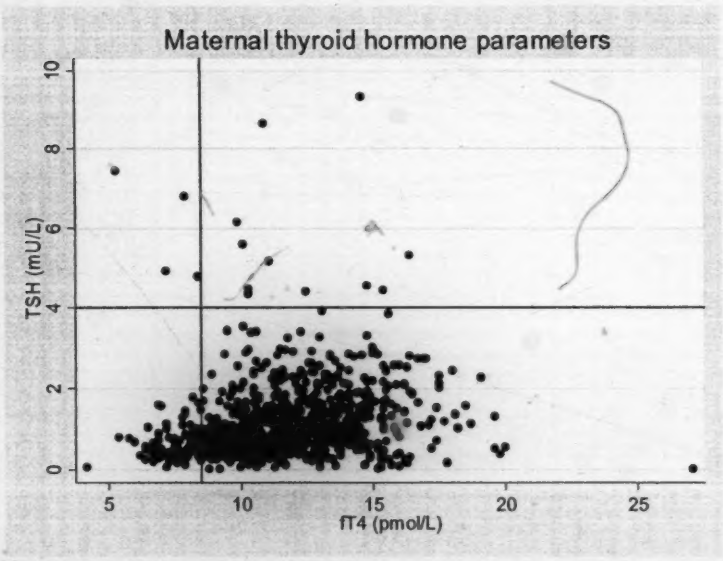

Figure 2.2. Maternal free thyroxine (fT_4) concentration by TSH concentration at the time of prenatal screening. The vertical line indicates the tenth percentile of fT_4 concentrations (8.5 pmol/L) and the horizontal line indicates the upper limit of the normal range of TSH (4.0 mU/L). Women in the lower left-hand quadrant are hypothyroxinemic, while those in the lower right-hand quadrant are euthyroid.

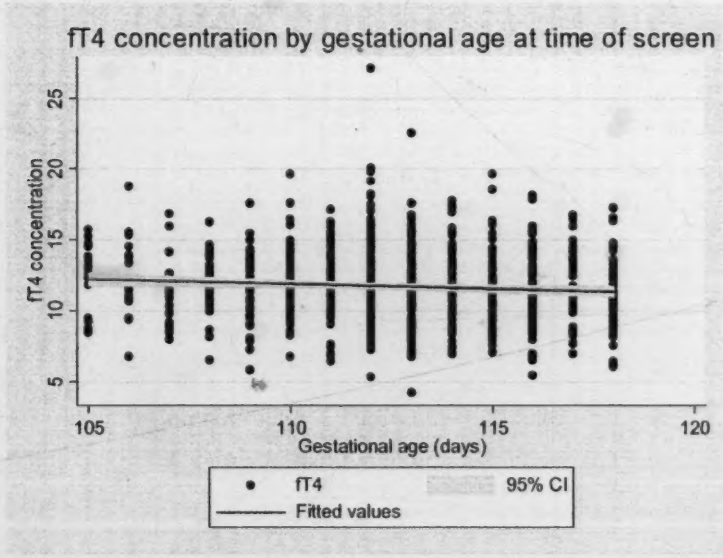

Figure 2.3. Maternal free thyroxine (fT_4) concentration by gestational age at the time of prenatal screening.

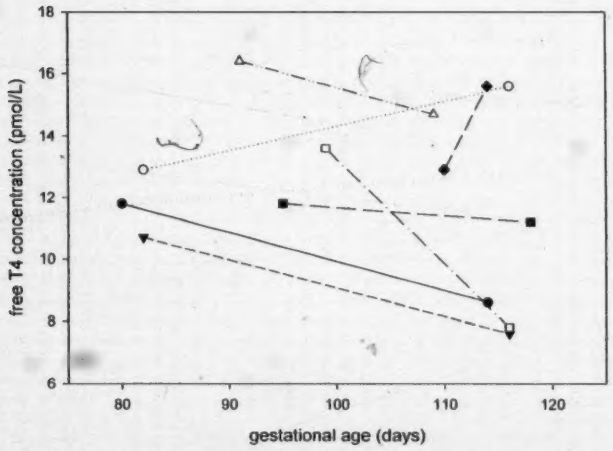

Figure 2.4. Subjects who underwent duplicate prenatal screens, resulting in two free thyroxine (T_4) measurements from two separate collection dates. Each line represents one study subject.

Table 2.2. Comparison of women not linked to those linked at the Alberta Perinatal Health Program.

Characteristic	Not linked (N=72)	p-value*	Linked (N=902)	Study subjects (N=879)	p-value*	All women screened (N=15,006)	p-value**
Maternal thyroid hormone status – N (%)						NA	NA
Euthyroid	62 (86.1%)	1.00	775 (85.9%)	756 (86.0%)	1.00		
Hypothyroxinemic	5 (6.9%)	0.54	91 (10.1%)	89 (10.1%)	1.00		
Hypothyroid	3 (4.2%)	0.14	15 (1.7%)	15 (1.7%)	1.00		
Hyperthyroid	2 (2.8%)	0.69	21 (2.3%)	19 (2.2%)	0.87		
Maternal age – mean (SD)	28.7 (6.9)	<0.01	30.6 (4.8)	30.6 (4.8)	1.00	29.8 (5.3)	<0.01
Maternal age – N (%)							
<20	11 (15.3%)	<0.01	16 (1.8%)	16 (1.8%)	1.00	518 (3.5)	0.01
20-24	14 (19.4%)	0.05	99 (11.0%)	98 (11.2%)	0.94	2332 (15.5)	<0.01
25-29	12 (16.7%)	0.02	275 (30.5%)	267 (30.3%)	1.00	4725 (31.5)	0.50
30-34	21 (29.2%)	0.16	342 (37.9%)	334 (38.0%)	1.00	4804 (32.0)	<0.01
35-39	12 (16.7%)	1.00	153 (17.0%)	147 (16.7%)	0.90	2292 (15.3)	0.25
≥40	2 (2.8%)	0.65	17 (1.9%)	17 (1.9%)	1.00	335 (2.2)	0.64
Maternal race – N (%)							
Caucasian	50 (69.4%)	0.70	600 (66.5%)	589 (67.0%)	0.84	10,113 (67.4)	0.82
Asian	7 (9.7%)	0.71	109 (12.1%)	108 (11.7%)	0.94	1442 (9.6)	0.01
Hispanic	0 (0.0%)	1.00	7 (0.8%)	7 (0.8%)	1.00	88 (0.6)	0.37
Black	2 (2.8%)	0.33	14 (1.6%)	14 (1.6%)	1.00	277 (1.9)	0.70
Semitic	0 (0.0%)	1.00	5 (0.6%)	5 (0.6%)	1.00	76 (0.5)	0.81
Filipino	1 (1.4%)	1.00	12 (1.3%)	11 (1.3%)	1.00	89 (0.6)	0.03
First Nation	0 (0.0%)	0.62	14 (1.6%)	13 (1.5%)	1.00	563 (3.8)	<0.01
Oriental	3 (4.2%)	1.00	47 (5.2%)	47 (5.4%)	0.92	601 (4.0)	1.00
Korean	0 (0.0%)	1.00	0 (0.0%)	0 (0.05%)	1.00	2 (0.01)	0.05
Unknown	9 (12.5%)	0.55	94 (10.4%)	90 (10.2%)	0.94	1755 (11.7)	0.21
Gestational age at test (days) – mean (SD)	112.5 (3.3)	0.79	112.6 (3.1)	112.6 (3.1)	1.00	116.6 (13.8)	<0.01
Month of prenatal screen – N (%)							
January	12 (16.7%)	0.87	167 (18.5%)	161 (18.3%)	0.95	1897 (12.6%)	<0.01
February	14 (19.4%)	0.63	153 (17.0%)	151 (17.2%)	0.95	1534 (10.2%)	<0.01
March	2 (2.8%)	0.76	38 (4.2%)	37 (4.2%)	1.00	939 (6.3%)	0.01
April	14 (19.4%)	0.41	142 (15.7%)	140 (15.9%)	0.95	1008 (6.7%)	<0.01
May	16 (22.2%)	0.53	171 (19.0%)	164 (18.7%)	0.90	1022 (6.8%)	<0.01
June	6 (8.3%)	0.21	126 (14.0%)	123 (14.0%)	1.00	942 (6.3%)	<0.01
July	NA	NA	NA	NA	NA	906 (6.0%)	NA
August	NA	NA	NA	NA	NA	1002 (6.7%)	NA
September	NA	NA	NA	NA	NA	1299 (8.7%)	NA
October	NA	NA	NA	NA	NA	1451 (9.7%)	NA
November	NA	NA	NA	NA	NA	1594 (10.6%)	NA
December	8 (11.1%)	1.00	105 (11.6%)	103 (11.7%)	1.00	1412 (9.4%)	0.03

*p-values calculated with the linked group (N=902) as the reference

**p-values calculated with the study subjects (N=879) as the reference

NA: not applicable

Table 2.3. Comparison of the study cohort to women in Alberta.

Characteristic	*Study subjects*	*Alberta women*
Mean maternal age (years)	30.6	29.0
Premature delivery (<37 weeks)	7.5%	9.1%
Smokers	11.0%	25.7%
Alcohol consumption	0.6%	18.0%

With data from (19).

Table 2.4. Measures of fetal growth and neonatal morbidity, according to infant and maternal variables.

Characteristic	*N (%)*	*Mean birth weight (SD) - grams*	*Mean z-score (SD)*	*Small for gestational age N (%)*	*Small for gestational age RR (95% CI)*	*Apgar <7 N(%)*	*Apgar <7 RR (95% CI)*
Total population	879 (100.0)	3378 (523)	0.076 (0.95)	61 (6.9)	-	9 (1.0)	-
Infant variables							
Gender							
Female	452 (51.4)	3305 (484)	0.023 (0.94)	35 (7.7)	1.0	3 (0.7)	1.0
Male	427 (48.6)	3455 (550)*	0.13 (0.95)†	26 (6.1)	0.79 (0.48–1.28)	6 (1.4)	2.12 (0.53-8.42)
Preterm delivery							
No	813 (92.5)	3439 (465)	0.068 (0.94)	56 (6.9)	1.0	7 (0.9)	1.0
Yes	66 (7.5)	2628 (607)*	0.18 (0.97)	5 (7.6)	1.1 (0.46-2.65)	2 (3.0)	3.52 (0.75-16.62)
Suspected anomaly							
No	877 (99.8)	3378 (523)	0.075 (0.95)	61 (7.0)	1.0	9 (1.0)	1.0
Yes	2 (0.2)	3550 (608)	0.31 (0.64)	0 (0.0)	-	0 (0.0)	-
Maternal variables							
Thyroid hormone status							
Euthyroid	756 (86.0)	3373 (518)	0.055 (0.92)	58 (7.7)	1.0	9 (1.2)	1.0
Hypothyroxinemic	89 (10.1)	3402 (503)	0.16 (0.91)	2 (2.3)†	0.29 (0.07-1.18)	0 (0.0)	-
Hypothyroid	15 (1.7)	3527 (590)	0.52 (1.1)†	0 (0.0)	-	0 (0.0)	-
Hyperthyroid	19 (2.2)	3342 (724)	0.18 (1.6)	1 (5.3)	0.69 (0.10-4.70)	0 (0.0)	-
Maternal age							
<20	16 (1.8)	3499 (624)	0.15 (1.1)	3 (18.8)†	3.68 (1.20-11.30)	1 (6.3)	10.43 (1.00-109.38)
20-24	98 (11.2)	3307 (607)	-0.090 (1.0)*	13 (13.3)*	2.61 (1.31-5.18)	1 (1.0)	1.70 (0.16-18.62)
25-29	267 (30.4)	3365 (520)	0.57 (0.99)	17 (6.4)	1.25 (0.65-2.40)	4 (1.5)	2.50 (0.46-13.57)
30-34	334 (38.0)	3398 (488)	0.12 (0.89)	17 (5.1)	1.0	2 (0.6)	1.0
35-39	147 (16.7)	3382 (543)	0.097 (0.91)	10 (6.8)	1.34 (0.63-2.85)	1 (0.7)	1.14 (0.10-12.45)
≥40	17 (1.9)	3458 (408)	0.17 (0.90)	1 (5.9)	1.16 (0.16-8.19)	0 (0.0)	-

* p<0.05
† p<0.10

Characteristic	*N (%)*	*Mean birth weight (SD) - grams*	*Mean z-score (SD)*	*Small for gestational age N (%)*	*Small for gestational age RR (95% CI)*	*Apgar <7 N (%)*	*Apgar <7 RR (95% CI)*
Maternal race							
Caucasian	589 (67.0)	3434 (505)	0.17 (0.94)	30 (5.1)	1.0	5 (0.9)	1.0
Asian	103 (11.7)	3197 (538)*	-0.28 (0.99)*	14 (13.6)*	2.67 (1.47-4.86)	2 (1.9)	2.29 (0.45-11.64)
Hispanic	7 (0.8)	3227 (560)	-0.13 (1.2)	1 (14.3)	2.81 (0.44-17.81)	0 (0.0)	-
Black	14 (1.6)	3295 (443)	-0.0036 (1.1)	2 (14.3)	2.81 (0.74-10.61)	0 (0.0)	-
Semitic	5 (0.6)	3292 (348)	-0.23 (0.81)	1 (20.0)	3.93 (0.66-23.48)	0 (0.0)	-
Filipino	11 (1.3)	3252 (576)	-0.0014 (0.69)	1 (9.1)	1.79 (0.27-11.96)	0 (0.0)	-
First Nation	13 (1.5)	3114 (796)*	0.26 (0.51)	0 (0.0)	-	1 (7.7)	9.06 (1.14-72.29)
Oriental	47 (5.4)	3281 (436)*	-0.19 (0.76)*	4 (8.5)	1.67 (0.61-4.54)	0 (0.0)	-
Unknown	90 (10.2)	3352 (568)	0.060 (0.92)	8 (8.9)	1.75 (0.83-3.69)	1 (1.1)	1.31 (0.15-11.09)
Gravida							
1	292 (33.2)	3361 (536)	-0.0088 (0.96)	26 (8.9)	1.0	3 (1.0)	1.0
2	288 (32.8)	3363 (530)	0.016 (0.93)	17 (5.9)	0.66 (0.37-1.20)	4 (1.4)	1.35 (0.31-5.99)
3	180 (20.5)	3407 (515)	0.20 (0.97)*	11 (6.1)	0.69 (0.35-1.36)	1 (0.6)	0.54 (0.06-5.17)
4-12	119 (13.5)	3413 (484)	0.24 (0.89)*	7 (5.9)	0.66 (0.29-1.48)	1 (0.8)	0.82 (0.09-7.79)
Weight >91kg							
No	792 (90.1)	3362 (516)	0.032 (0.93)	58 (7.3)	1.0	9 (1.1)	1.0
Yes	87 (9.9)	3524 (564)*	0.48 (1.0)*	3 (3.5)	0.47 (0.15-1.47)	0 (0.0)	-
Weight <45kg							
No	877 (99.8)	3379 (523)	0.078 (0.94)	60 (6.8)	1.0	9 (1.0)	1.0
Yes	2 (0.2)	3018 (237)	-0.65 (1.6)	1 (50.0)	7.3 (1.79-29.88)	0 (0.0)	-
Height <152cm							
No	863 (98.2)	3381 (523)	0.082 (0.95)	57 (6.6)	1.0	9 (1.0)	1.0
Yes	16 (1.8)	3247 (492)	-0.27 (0.92)	4 (25.0)*	3.79 (1.56-9.18)	0 (0.0)	-
Poor weight gain							
No	872 (99.2)	3380 (521)	0.080 (0.94)	59 (6.8)	1.0	9 (1.0)	1.0
Yes	7 (0.8)	3145 (695)	-0.41 (1.3)	2 (28.6)†	4.22 (1.27-13.99)	0 (0.0)	-
Previous SGA delivery							
No	871 (99.1)	3383 (520)	0.086 (0.94)	58 (6.7)	1.0	8 (0.9)	1.0
Yes	8 (0.9)	2899 (572)*	-1.0 (0.98)*	3 (37.5)*	5.63 (2.22-14.26)	1 (12.5)†	13.61 (1.92-96.61)
Pre-existing diabetes							
No	861 (98.0)	3383 (518)	0.064 (0.94)	60 (7.0)	1.0	9 (1.1)	1.0
Yes	18 (2.1)	3152 (684)†	0.67 (1.1)*	1 (5.6)	0.80 (0.12-5.45)	0 (0.0)	-

* $p<0.05$
† $p<0.10$

Characteristic	*N (%)*	*Mean birth weight (SD) - grams*	*Mean z-score (SD)*	*Small for gestational age N (%)*	*Small for gestational age RR (95% CI)*	*Apgar <7 N (%)*	*Apgar <7 RR (95% CI)*
Gestational diabetes							
No	832 (94.7)	3381 (528)	0.069 (0.95)	59 (7.1)	1.0	9 (1.1)	1.0
Yes	47 (5.4)	3331 (414)	0.20 (0.80)	2 (4.3)	0.60 (0.15-2.38)	0 (0.0)	-
Hypertension (HTN)							
No	869 (98.9)	3379 (521)	0.072 (0.95)	61 (7.0)	1.0	9 (1.0)	1.0
Yes	10 (1.1)	3281 (688)	0.39 (0.57)	0 (0.0)	-	0 (0.0)	-
Pregnancy-induced HTN							
No	837 (95.2)	3388 (513)	0.078 (0.94)	58 (6.9)	1.0	9 (1.1)	1.0
Yes	42 (4.8)	3182 (659)*	0.039 (0.98)	3 (7.1)	1.03 (0.34-3.16)	0 (0.0)	-
Proteinuria							
No	870 (99.0)	3383 (520)	0.080 (0.95)	59 (6.8)	1.0	9 (1.0)	1.0
Yes	9 (1.0)	2886 (540)*	-0.30 (0.90)	2 (22.2)	3.28 (0.94-11.41)	0 (0.0)	-
Preeclampsia							
No	871 (99.1)	3384 (520)	0.081 (0.95)	59 (6.8)	1.0	9 (1.0)	1.0
Yes	8 (0.9)	2769 (441)*	-0.43 (0.86)	2 (25.0)†	3.69 (1.08-12.58)	0 (0.0)	-
Chronic renal disease							
No	876 (99.7)	3382 (520)	0.076 (0.95)	61 (7.0)	1.0	9 (1.0)	1.0
Yes	3 (0.3)	2418 (288)*	-0.023 (0.34)	0 (0.0)	-	0 (0.0)	-
Smoker							
No	782 (89.0)	3396 (517)	0.093 (0.95)	51 (6.5)	1.0	6 (0.8)	1.0
Yes	97 (11.0)	3236 (548)*	-0.060 (0.92)	10 (10.3)	1.58 (0.83-3.01)	3 (3.1)†	4.03 (1.02-15.87)
Alcohol							
No	874 (99.4)	3378 (524)	0.077 (0.95)	61 (7.0)	1.0	8 (0.9)	1.0
Yes	5 (0.6)	3480 (264)	-0.10 (0.36)	0 (0.0)	-	1 (20.0)*	21.85 (3.32-143.90)
Drug-dependent							
No	873 (99.3)	3379 (524)	0.077 (0.95)	61 (7.0)	1.0	9 (1.0)	1.0
Yes	6 (0.7)	3338 (338)	-0.10 (0.79)	0 (0.0)	-	0 (0.0)	-

* $p<0.05$
† $p<0.10$

Table 2.5. Estimates of fetal growth restriction due to hypothyroxinemia, adjusted for potential confounding factors.

	Poisson regression: Small for gestational age (SGA)			*Linear regression: Z-score of birth weight for gestational age and gender*	
Variable	*N(SGA)*	*N(births)*	*Relative Risk (95% CI)*	*β coefficient (95% CI) – full population*	*β coefficient (95% CI) – full term births*
Euthyroid	58	756	1.00	-1.00 (-2.92, 0.92)	-0.68 (-2.69, 1.33)
Hypothyroxinemic	2	89	0.38 (0.11, 1.33)	0.035 (-0.17, 0.24)	0.013 (-0.20, 0.22)
Hypothyroid	0	15	*	0.48 (0.006, 0.95)	0.35 (-0.16, 0.86)
Hyperthyroid	1	19	0.66 (0.10, 4.21)	0.13 (-0.29, 0.56)	0.061 (-0.42, 0.54)
Suspected anomaly	0	2	*	0.23 (-1.13, 1.58)	0.27 (-1.09, 1.63)
Maternal age	-	-	0.55 (0.36, 0.84)	0.069 (-0.058, 0.20)	0.048 (-0.08, 0.18)
Maternal age squared	-	-	1.01 (1.00, 1.02)	-0.0011 (-0.003, 0.001)	-0.00073 (-0.003, 0.001)
Maternal race					
Caucasian	30	589	1.00	-1.00 (-2.92, 0.92)	-0.68 (-2.69, 1.33)
Asian	14	103	3.31 (1.76, 6.21)	-0.47 (-0.66, -0.27)	-0.48 (-0.68, -0.27)
Hispanic	1	7	3.28 (0.48, 22.52)	-0.27 (-0.98, 0.43)	-0.14 (-0.96, 0.68)
Black	2	14	2.69 (0.66, 11.02)	-0.19 (-0.67, 0.30)	-0.21 (-0.72, 0.29)
Semitic	1	5	5.88 (0.93, 37.31)	-0.58 (-1.39, 0.23)	-0.59 (-1.41, 0.22)
Filipino	1	11	2.52 (0.34, 18.54)	-0.17 (-0.72, 0.38)	-0.20 (-0.78, 0.38)
First Nation	0	13	*	0.12 (-0.39, 0.64)	0.22 (-0.36, 0.81)
Oriental	4	47	2.02 (0.85, 4.80)	-0.37 (-0.65, -0.09)	-0.39 (-0.68, -0.09)
Unknown	8	90	1.68 (0.81, 3.47)	-0.074 (-0.28, 0.13)	-0.058 (-0.28, 0.16)
Gravida					
1	26	292	1.00	-1.00 (-2.92, 0.92)	-0.68 (-2.69, 1.33)
2	17	288	0.81 (0.41, 1.57)	0.017 (-0.14, 0.17)	0.0089 (-0.15, 0.17)
3	11	180	0.81 (0.38, 1.73)	0.19 (0.009, 0.36)	0.17 (-0.01, 0.36)
≥4	7	119	0.82 (0.34, 1.99)	0.29 (0.08, 0.50)	0.27 (0.05, 0.49)
Weight >91 kg	3	87	0.65 (0.22, 1.97)	0.36 (0.15, 0.58)	0.39 (0.16, 0.61)
Weight <45 kg	1	2	3.04 (1.10, 8.38)	-0.52 (-1.82, 0.79)	-0.51 (-1.82, 0.80)
Height <152 cm	4	16	1.83 (0.74, 4.57)	-0.023 (-0.50, 0.46)	-0.0037 (-0.51, 0.50)
Poor weight gain	2	7	2.86 (1.35, 6.05)	-0.70 (-1.40, -0.003)	-0.58 (-1.33, 0.18)
Previous SGA delivery	3	8	8.54 (3.01, 24.18)	-1.07 (-1.73, -0.42)	-1.08 (-1.74, -0.42)
Pre-existing diabetes	1	18	1.17 (0.12, 11.27)	0.46 (0.014, 0.91)	0.13 (-0.53, 0.78)
Gestational diabetes	2	47	0.53 (0.13, 2.26)	0.12 (-0.15, 0.40)	0.047 (-0.26, 0.35)
Hypertension	0	10	*	0.10 (-0.60, 0.81)	0.15 (-0.61, 0.91)
Preeclampsia	2	8	5.85 (1.82, 18.78)	-0.71 (-1.36, -0.05)	-0.52 (-1.34, 0.29)
Chronic renal disease	0	3	0.21 (0.03, 1.46)	-0.037 (-1.34, 1.26)	*
Smoker	10	97	2.02 (0.92, 4.44)	-0.25 (-0.46, 0.04)	-0.25 (-0.48, -0.02)
Any alcohol consumption during pregnancy	0	5	*	-0.051 (-0.88, 0.78)	-0.069 (-0.91, 0.77)
Drug dependence	0	6	*	-0.083 (-0.89, 0.72)	-0.083 (-0.89, 0.73)

*Estimates were unable to be obtained in cells with no observations

Table 2.6. Estimates of fetal growth restriction and early neonatal morbidity due to free thyroxine concentration (pmol/L), adjusted for potential confounding factors.

Variable	*SGA – Relative Risk (95% CI)*	*Z-score – B-coefficient (95% CI)*		*Apgar <7 – Relative Risk (95% CI)*
		Full population	*Full term births*	
Free T_4	1.29 (0.69, 2.43)	0.048 (-0.14, 0.24)	0.082 (-0.11, 0.28)	3.04 (0.80, 11.48)
TSH	0.71 (0.53, 0.94)	0.084 (0.02, 0.15)	0.082 (0.01, 0.15)	0.86 (0.51, 1.46)
Free T_4 * Gestational age at test	1.00 (0.99, 1.00)	-0.00044 (-0.002, 0.001)	-0.0007 (-0.002, 0.001)	0.99 (0.98, 1.00)
Maternal age	0.54 (0.36, 0.80)	0.076 (-0.05, 0.20)	0.055 (-0.08, 0.19)	1.43 (0.08, 25.44)
Maternal age squared	1.01 (1.00, 1.02)	-0.0012 (-0.003, 0.0009)	-0.00083 (-0.003, 0.001)	0.99 (0.95, 1.04)
Infant gender (male)	NA	NA	NA	1.49 (0.48, 4.62)
Gestational age	NA	NA	NA	0.023 (0.0005, 0.99)
Gestational age squared	NA	NA	NA	1.05 (0.99, 1.12)
Suspected anomaly	*	0.15 (-1.20, 1.50)	0.20 (-1.15, 1.56)	*
Maternal race				
Caucasian	1.00	-1.18 (-3.14, 0.77)	-0.90 (-2.95, 1.15)	1.00
Asian	3.08 (1.63, 5.85)	-0.46 (-0.66, -0.26)	-0.48 (-0.68, -0.27)	1.68 (0.19, 14.87)
Hispanic	3.70 (0.54, 25.32)	-0.29 (-1.00, 0.41)	-0.12 (-0.94, 0.69)	*
Black	2.75 (0.68, 11.18)	-0.15 (-0.64, 0.34)	-0.17 (-0.68, 0.34)	*
Semitic	5.81 (0.87, 38.70)	-0.65 (-1.46, 0.16)	-0.67 (-1.49, 0.15)	*
Filipino	2.25 (0.28, 18.11)	-0.18 (-0.74, 0.37)	-0.22 (-0.80, 0.36)	*
First Nation	*	0.16 (-0.36, 0.67)	0.26 (-0.32, 0.84)	5.77 (0.70, 47.54)
Oriental	1.79 (0.76, 4.24)	-0.36 (-0.64, -0.07)	-0.38 (-0.67, -0.08)	*
Unknown	1.69 (0.85, 3.35)	-0.074 (-0.28, 0.13)	-0.065 (-0.28, 0.15)	0.61 (0.14, 2.76)
Gravida				
1	1.00	-1.18 (-3.14, 0.77)	-0.90 (-2.95, 1.15)	1.00
2	0.78 (0.40, 1.52)	0.024 (-0.13, 0.18)	0.012 (-0.15, 0.17)	0.80 (0.13, 4.99)
3	0.76 (0.36, 1.63)	0.19 (0.01, 0.36)	0.17 (-0.01, 0.36)	0.60 (0.03, 13.12)
≥4	0.86 (0.36, 2.04)	0.29 (0.08, 0.50)	0.26 (0.04, 0.48)	0.42 (0.09, 1.98)
Weight >91 kg	0.62 (0.22, 1.79)	0.36 (0.15, 0.57)	0.38 (0.16, 0.60)	*
Weight <45 kg	4.85 (1.61, 14.63)	-0.66 (-1.96, 0.65)	-0.65 (-1.96, 0.66)	*
Height <152 cm	1.70 (0.62, 4.64)	-0.011 (-0.49, 0.47)	0.011 (-0.49, 0.51)	*
Poor weight gain	2.14 (0.93, 4.91)	-0.67 (-1.36, 0.03)	-0.56 (-1.31, 0.19)	*
Previous SGA delivery	8.18 (2.89, 23.17)	-1.06 (-1.71, -0.40)	-1.06 (-1.71, -0.40)	8.17 (2.01, 33.16)
Pre-existing diabetes	0.98 (0.08, 11.97)	0.48 (0.04, 0.93)	0.10 (-0.55, 0.76)	*
Gestational diabetes	0.50 (0.11, 2.19)	0.13 (-0.14, 0.41)	0.052 (-0.25, 0.36)	*
Hypertension	*	0.094 (-0.61, 0.80)	0.17 (-0.59, 0.93)	*
Preeclampsia	6.33 (2.10, 19.09)	-0.72 (-1.37, -0.06)	-0.51 (-1.32, 0.30)	*
Chronic renal disease	0.23 (0.03, 1.57)	0.041 (-1.35, 1.26)	*	*
Smoker	1.86 (0.83, 4.16)	-0.23 (-0.44, -0.02)	-0.24 (-0.46, -0.005)	3.95 (0.29, 53.93)
Any alcohol consumption during pregnancy	*	-0.054 (-0.88, 0.78)	-0.080 (-0.91, 0.76)	22.36 (2.60, 192.11)
Drug dependence	*	-0.062 (-0.87, 0.74)	-0.052 (-0.86, 0.76)	*

NA: not applicable

*Estimates were unable to be obtained in cells with no observations

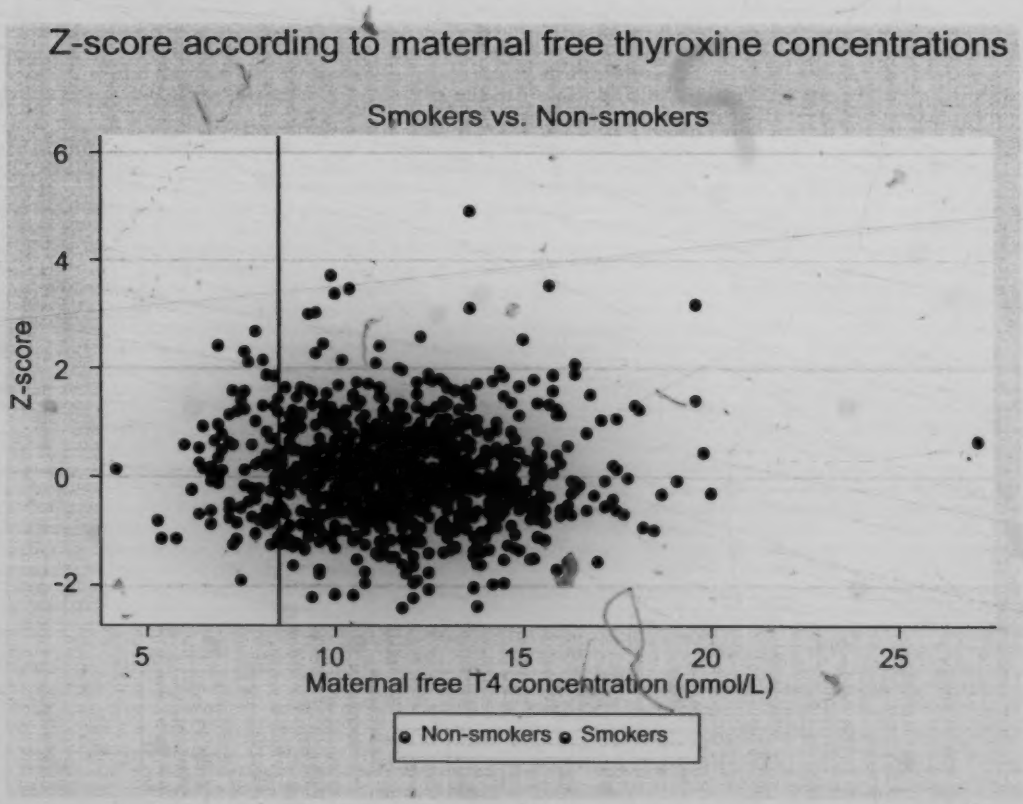

Figure 2.5. Infant z-score according to maternal free thyroxine (fT_4) concentration (pmol/L), stratified by maternal smoking status. The vertical line indicates the tenth percentile for maternal fT_4 concentrations (8.5 pmol/L).

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Chapter 3
Relevance to Environmental Health and Future Research

3.1. Overview

The goal of this thesis was to determine whether a correlation exists between maternal hypothyroxinemia and fetal growth restriction and/or early neonatal morbidity. Using a prospective cohort of 879 women and their babies, no association was found between a maternal deficiency of free T_4 and the delivery of an infant that was small for gestational age, that had a reduced z-score for birth weight, or that had a five minute Apgar score less than 7. Although these findings do not support the hypothesized association, they should serve as reassurance that subclinical maternal thyroid hormone deficiencies do not appear to increase the risk of these adverse perinatal outcomes.

3.2. Relevance to environmental health research

The research objectives for this study were largely drawn from evidence collected in developmental toxicity studies related to environmental exposures to endocrine disruptors. In a list published in 2002, 116 chemicals were identified that interfered with normal thyroid function through disruptions of hormone production, transport, and metabolism (1). While this list was not intended to be exhaustive, it still highlights the sensitivity of the thyroid to synthetic compounds present in the environment. Some of the compounds considered to be thyroid toxicants are the widespread environmental contaminants polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and polybrominated biphenyls (PBBs) (2). Of these, PCBs are the most widely studied, and it is recognized that exposure to these chemicals is associated with reduced circulating T_4 in animal models, and possibly with hypothyroidism (2-5). Reductions in IQ, impaired visual recognition memory, and deficits in attention and motor ability have been correlated with PCB body burden (2), all of which are also associated with thyroid hormone deficiencies. Although less is known about PBDEs and PBBs, they are structurally related to PCBs, making it possible that their pattern of toxicity mimics that of PCBs.

Another class of chemical that has been linked to disruption of thyroid hormone homeostasis is perfluorinated acids (PFAs). These are chemicals that have been widely used in industry and in consumer products for decades, and have recently been recognized as persistent organic pollutants with potentially toxic effects on development (6). In animal models, exposure to perfluorooctane sulfonate (PFOS), an eight-carbon member of the PFA family, has been correlated with a reduction in both maternal and fetal levels of circulating thyroid hormones, while TSH levels remain constant (6,7). Perfluorooctanoic acid (PFOA), another widespread member of the class, has also been linked with a disruption of free thyroxine levels (8). One of the most consistently seen toxicities related to PFA exposure is a reduction in fetal weight at birth, which has been suggested to be the result of thyroid hormone disruption, potentially via interference with cellular or functional maturation of organ systems (7). Both PFOS and PFOA are able to cross the placenta (7,9,10), therefore if the observed toxicities are mediated by thyroid hormones, it is unknown whether they are the result of a maternal thyroid deficiency, or of direct fetal exposure to the compounds, leading to an isolated fetal thyroid deficiency.

At this point, there is no epidemiological evidence linking PFA exposure to hypothyroxinemia. However, in a study currently underway at the University of Alberta, that relationship is being examined[1]. The serum samples that are being analyzed for PFA and thyroid hormone concentrations were collected from women belonging to the same cohort as was used in the present study. As such, once the maternal PFA concentrations have been determined, an intended follow-up study will be to link these numbers to the pregnancy outcome data already collected, to investigate whether PFA exposure is associated with adverse birth outcomes, and if this is the case, whether this pathway is mediated by thyroid hormone levels. This data will hopefully build on the small number of studies that have looked at the association between maternal PFA exposure and fetal growth restriction (11-13). In the first, Grice *et al.* conducted a survey of an occupational cohort exposed to PFOS, including questions on pregnancy-related information (11). Of

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[1] Emerging contaminants as determinants of maternal hypothyroxinaemia in Edmonton, Alberta
Source: University (of Alberta) Hospital Foundation
PI: JW Martin
Co-Inv: I Burstyn, F Bamforth, NM Cherry

the 263 women surveyed, 421 live births were reported. Among these births, there were no appreciable differences in birth weight among PFOS exposure categories. Apelberg *et al.* collected 293 cord serum samples between 2004 and 2005 from a single hospital in Baltimore, Maryland (12). Analysis for PFOS and PFOA concentrations was conducted, and these levels were linked to maternal characteristics and birth outcomes. Modest reductions in birth weight were observed, although these were non-significant: PFOS (median concentration 5 ng/mL, range <LOD (0.2) – 34.8 ng/mL) was associated with a decrease of 69 g per ln-unit (95% CI -149 to 10 g) and PFOA (median 1.6 ng/mL, range 0.3 – 7.1 ng/mL) was associated with a decrease of 104 g (95% CI -213 to 5 g) after adjustment for potential confounders. Finally, Fei *et al.* employed a prospective cohort comprised of 1,399 women and their infants selected from the Danish National Birth Cohort (1996-2002) to examine the association between maternal PFOS and PFOA concentrations and birth weight and length of gestation (13). As in the study described above, a slight reduction in birth weight (-10.63 g per 1ng/mL; 95% CI -20.79 to -0.47 g) was observed with increasing levels of PFOA (mean concentration 5.6 ng/mL), however no association was found with PFOS (mean 35.3 ng/mL). Similarly, there was no relationship between PFA levels and the incidence of low birth weight (<2,500 g), small for gestational age (birth weight <10th percentile for gestational age and infant gender), or preterm birth (<37 weeks gestation).

This presentation of some of the available data relating to the effects of PFAs on thyroid function and developmental toxicity only represents a small fraction of the toxicological evidence supporting a role for endocrine disruptors in the environment in the disruption of thyroid hormone homeostasis. As mentioned above, there are numerous compounds released into the environment that may act via the same mechanism, making it difficult to isolate the effects that may be specific to exposures to PFAs, or to any other single chemical class. However, benefits can be derived from knowing how these chemicals act as a group, especially since it is likely that real-world exposure will involve a mixture of chemicals. If it is determined that thyroid toxicants can exert their effects through an induction of hypothyroxinemia, it is advantageous to know of any adverse effects associated with that condition, which can then be generalized to a number of compounds.

Conversely, if the association between hypothyroxinemia and potential toxicities is refuted, concern regarding a whole group of compounds can be reduced. Since PFAs and many other thyroid toxicants are widespread in the environment, using one chemical, group of chemical, or a known consequence of chemical exposure as markers of exposure to a wider group of compounds is likely an efficient method of measuring the overall manifest effects of the group as a whole.

3.3. Significance and future research

The findings of this study do not provide evidence that supports an adverse relationship between maternal hypothyroxinemia and pregnancy outcomes. By extension, if environmental contaminants are associated with restricted fetal growth or early neonatal morbidity, it is unlikely that this relationship is mediated by maternal thyroid hormone levels. These findings can serve as reassurance, both at the levels of clinical and of public health significance.

An ongoing debate related to hypothyroxinemia is whether this condition is associated with outcomes severe enough to warrant widespread free T_4 screening among pregnant women (14,15). It is known from research and from experience with hypothyroidism that treatment with thyroxine is highly beneficial in the prognosis of the developing fetus, especially when initiated early in pregnancy, an element that would be facilitated by early detection of disease (16). Currently, screening procedures are limited to women with overt symptoms, or to those in whom a reasonable suspicion of thyroid disruption exists (17), something that has not been extended to hypothyroxinemia. As this is a subclinical condition without obvious manifestations, the screening procedure would have to be universal. While studies related to neurological development are in support of the implementation of widespread screening programs, this study did not find a marked effect on fetal growth that would provide evidence to further this initiative.

There remains much to be learned about the effect of maternal hypothyroxinemia on fetal development. This study provided a preliminary look at the outcomes of fetal growth restriction and early neonatal morbidity, but there has also been speculation about such

conditions as breech presentation, attention-deficit/hyperactivity disorder, and autism that warrant further research (18-20). In terms of fetal growth though, the focus of this study could also be applied in expanded and improved forums. The findings are based on a relatively homogenous population from one urban center. Even through the expansion of the study protocol to include data collected from the province of Alberta, the findings could be more informative. This could encompass a more diverse population, including women from rural areas, which could have a marked effect on factors ranging from behaviors (e.g. smoking rates) to maternal ethnicity (e.g. inclusion of more First Nations women). If it can be established in further studies that maternal hypothyroxinemia can be placed on the causal pathway in the manifestation of adverse fetal outcomes, it will also be important to determine if the benefits of thyroxine treatment that are observed in hypothyroid women and their infants can be experienced in this subclinical population as well.

An advantage of the current study was that maternal thyroid hormones were measured during a two-week window during gestation, limiting the data collected to 15-16 weeks gestation. This enabled a description of the effects of maternal hypothyroxinemia on fetal growth at a time prior to the onset of fetal thyroid function. However, free T_4 levels are not static during pregnancy, and they are known to decrease over the course of gestation (21). If free T_4 levels were measured throughout pregnancy, it could be determined if there was a crucial timeframe in which adverse effects may be induced, or if they may depend on a continued worsening of the maternal condition, or if, unlike hypothyroidism, hypothyroxinemia does not become severe enough to alter global development, instead only influencing the more sensitive CNS development.

Another interesting question that could be answered is what happens to these children during infancy and childhood. From research on hypothyroidism, it is known that the thyroid is instrumental in guiding growth in the postnatal period (22), so perhaps this would also be observed in infants born to hypothyroxinemic women. Despite our findings that fetal growth is not influenced by this deficiency, it is possible that a latent effect exists and manifests during a later period.

3.4. Conclusion

In a number of recent studies, maternal hypothyroxinemia has been shown to be associated with impaired neurological development, setting the stage for further research on its effects on other perinatal outcomes. The impact of maternal hypothyroxinemia on fetal growth restriction and early neonatal morbidity was assessed in this study, with the findings not supporting a significant relationship between maternal thyroid hormone homeostasis and these selected outcomes. While maternal hypothyroxinemia is believed to adversely affect some aspects of fetal development, it is encouraging that this does not appear to extend to fetal growth restriction and early neonatal morbidity.

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Appendix I. Delivery Record

Delivery Record - Part One

Antenatal Risk Assessment

Part A - Pre-Pregnancy (circle if applicable)

Score		
1	Age ≤ 17 at delivery	
2	Age ≥ 35 at delivery	
1	Weight ≥ 91 kg	
1	Weight ≤ 45 kg	
1	Height < 152 cm	
	Diabetes	
1	Controlled by diet only	
3	Insulin used	
3	Retinopathy documented	
	Heart Disease	
1	Asymptomatic (no affect on daily living)	
3	Symptomatic (affects daily living)	
	Hypertension	
2	140/90 or greater	
3	Antihypertensive Drugs	
2	Chronic Renal Disease Documented	
1	OTHER medical disorders e.g. epilepsy, severe asthma, lupus, Crohn's disease	

Part B - Past Obstetrical History (circle if applicable)

Score	
3	Neonatal death(s)
3	Stillbirth(s)
1	Abortion between 12 to 20 weeks and under 500 grams birth weight
1	Delivery at 20 - 37 weeks
2	Cesarean section
1	Small for dates - 5th percentile
1	Large for dates - 95th percentile
1	RH isoimmunization - unaffected infant
3	RH isoimmunization - affected infant
1	Major cong. anomaly e.g. Chromosomal, Heart, CNS defects

Part C - Problems in Current Pregnancy (circle if applicable)

Score	
2	Diagnosis of large for dates
3	Diagnosis of small for dates
2	Polyhydramnios or oligohydramnios
3	Multiple pregnancy
3	Malpresentation (breech or transverse lie)
2	Membranes ruptured before 37 weeks
1	Bleeding < 20 weeks
3	Bleeding ≥ 20 weeks
2	Gestational hypertension
1	Proteinuria ≥ 1+
1	Gestational diabetes documented
3	Blood antibodies (Rh, Anti C, Anti K, etc.)
1	Anaemia (Hgb< 100 gm. per L)
1	Pregnancy ≥ 41 weeks
1	Poor weight gain (26 - 36 weeks<0.5kg/week or weight loss)
1	Smoker - anytime during pregnancy

Part D - Other Risk Factors (Note: Scores to be validated) (circle if applicable)

Score		
3	Major fetal anomaly	
3	Acute Medical Disorder (acute Asthma, Thyrotoxicosis, UTI, etc.)	
3	Cervical surgery	
	Substance use:	
3	Alcohol - ≥ 3 drinks on any one occasion during pregnancy	
3	Alcohol - ≥ 1 drink per day throughout pregnancy	
3	Drug dependent	

Total Antepartum Risk Score

Intrapartum Risk Assessment

Score	(circle if applicable)	
2	≤ 34 weeks	
1	35 - 36 weeks	
1	Meconium in labour	
1	Gestational hypertension	
1	Anemia	
1	Fever	
1	Fetal heart rate abnormalities	
1	Bleeding	
1	Ruptured membranes > 24 hrs.	
1	Seizures	
1	Coagulopathy	

Total Intrapartum Risk Score

Indications for Induction

(circle primary indication)

1. Significant A.P.H.
2. Suspect fetal compromise
3. Current intrauterine death
4. P.R.O.M.
5. Suspect I.U.G.R.
6. Gestational hypertension
7. Past history perinatal death
8. Diabetes
9. Gestational diabetes
10. Gestation ≥ 41 weeks
11. Suspect large for gestational age
12. Chronic essential hypertension
13. Social
14. Other, Specify _____

Operative Delivery (c/s, forceps, vacuum extraction)

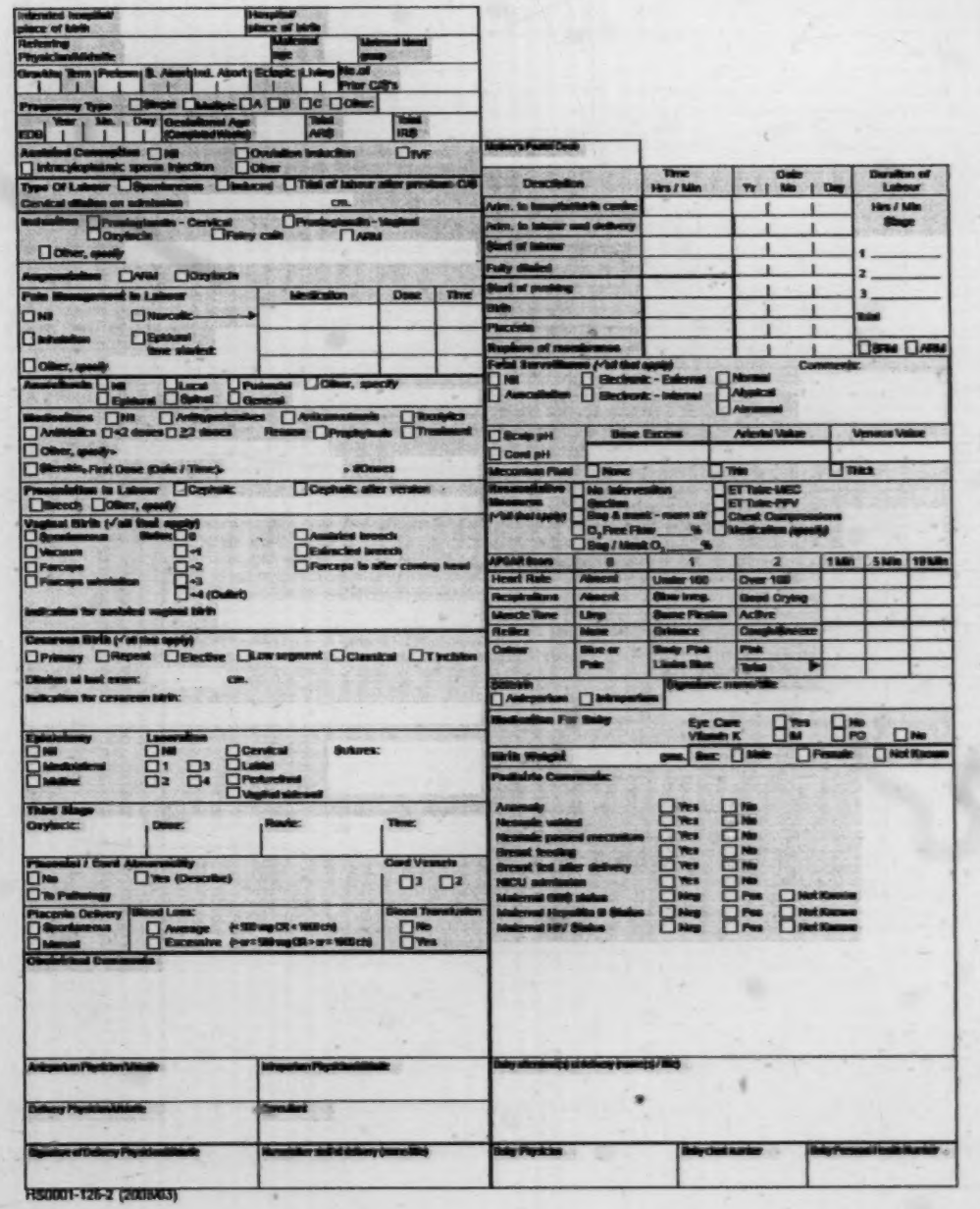
(circle primary indication)

1. Elective repeat c/s
2. Malpresentation (breech or transverse lie)
3. Arrest of progress in labor - first stage
4. Arrest of progress in labor - second stage
5. Failed trial of forceps
6. Fetal heart rate abnormalities
7. Intrapartum hemorrhage
8. Pyrexia in labor
9. Maternal hypertension
10. Maternal cardiac disease
11. Maternal endocrine disease (diabetes)
12. RH isoimmunization
13. Fetal malformation
14. Fetal illness (low platelets, etc.)
15. Multiple pregnancy
16. Prior hysterotomy
17. Placenta previa
18. Advanced maternal age
19. Maternal exhaustion
20. Maternal request
21. No medical reason
22. Other, specify _____

Date Signature

HS 0081-126-1 (2007/09)

Delivery Record - Part Two



Intended hospital/ place of birth | Hospital/ place of birth

Referring Physician/Midwife | Maternal age | Maternal blood group

Gravida | Term | Preterm | S. Aborts | Ind. Aborts | Ectopic | Living | No. of Prior C/S's

Pregnancy Type ☐ Single ☐ Multiple: ☐ A ☐ B ☐ C ☐ Other:

EDB: Year | Mo. | Day | Gestational Age (Completed Weeks) | Tubal ARB | Tubal IRB

Assisted Conception ☐ Nil ☐ Ovulation Induction ☐ IVF ☐ Intracytoplasmic sperm Injection ☐ Other

Type Of Labour ☐ Spontaneous ☐ Induced ☐ Trial of labour after previous C/S

Cervical dilation on admission: cm.

Induction ☐ Prostaglandin - Cervical ☐ Prostaglandin - Vaginal ☐ Oxytocin ☐ Foley cath ☐ ARM ☐ Other, specify

Augmentation ☐ ARM ☐ Oxytocin

Pain Management in Labour

Medication	Dose	Time

☐ Nil ☐ Narcotic ☐ Inhalation ☐ Epidural/ Spinal started ☐ Other, specify

Anesthesia ☐ Nil ☐ Local ☐ Pudendal ☐ Other, specify ☐ Epidural ☐ Spinal ☐ General

Medications ☐ Nil ☐ Antihypertensives ☐ Anticonvulsants ☐ Tocolytics ☐ Antibiotics ☐ <2 doses ☐ ≥2 doses Reason ☐ Prophylaxis ☐ Treatment ☐ Other, specify:

☐ Steroids, First Dose (Date / Time): > 48hours

Presentation in Labour ☐ Cephalic ☐ Cephalic after version ☐ Breech ☐ Other, specify

Vaginal Birth (✓all that apply)

☐ Spontaneous	Station ☐ 0	☐ Assisted breech
☐ Vacuum	☐ +1	☐ Extracted breech
☐ Forceps	☐ +2	☐ Forceps to after coming head
☐ Forceps rotation	☐ +3	
	☐ +4 (Outlet)	

Indication for assisted vaginal birth:

Cesarean Birth (✓all that apply) ☐ Primary ☐ Repeat ☐ Elective ☐ Low segment ☐ Classical ☐ T Incision

Dilation at last exam: cm.

Indication for cesarean birth:

Episiotomy ☐ Nil ☐ Mediolateral ☐ Midline

Laceration ☐ Nil ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ Cervical ☐ Labial ☐ Perineal ☐ Vaginal sidewall

Sutures:

Third Stage

Oxytocic: Dose: Route: Time:

Placenta / Cord Abnormality ☐ No ☐ Yes (Describe) ☐ To Pathology

Cord Vessels ☐ 3 ☐ 2

Placenta Delivery ☐ Spontaneous ☐ Manual

Blood Loss: ☐ Average (<=500 mg OR < 1000 cc) ☐ Excessive (>=500 mg OR > 1000 cc)

Blood Transfusion ☐ No ☐ Yes

Obstetrical Comments:

Antepartum Physician/Midwife | Intrapartum Physician/Midwife

Delivery Physician/Midwife | Resident

Signature of Delivery Physician/Midwife | Attendance at vaginal delivery (name/title)

Mother's Postnatal Desk

Description	Time Hrs / Min	Date Yr / Mo / Day	Duration of Labour
Adm. to hospital/birth centre			Hrs / Min Stage
Adm. to labour ward/ delivery			
Start of labour			1
Fully dilated			2
Start of pushing			3
Birth			Total
Placenta			
Rupture of membranes			☐ SRM ☐ ARM

Fetal Surveillance (✓all that apply) ☐ Nil ☐ Electronic - External ☐ Auscultation ☐ Electronic - Internal

Comments: ☐ Normal ☐ Atypical ☐ Abnormal

	Base Excess	Arterial Value	Venous Value
☐ Scalp pH			
☐ Cord pH			

Meconium Fluid ☐ None ☐ Thin ☐ Thick

Resuscitative Measures (✓all that apply) ☐ No Intervention ☐ Suction ☐ Bag & mask - room air ☐ O_2 Free Flow ____% ☐ Bag / Mask O_2 ____% ☐ ET Tube-MEC ☐ ET Tube-PPV ☐ Chest Compressions ☐ Medication (specify)

APGAR Score	0	1	2	1 Min	5 Min	10 Min
Heart Rate	Absent	Under 100	Over 100			
Respirations	Absent	Slow Irreg.	Good Crying			
Muscle Tone	Limp	Some Flexion	Active			
Reflex	None	Grimace	Cough/Sneeze			
Colour	Blue or Pale	Body Pink Limbs Blue	Pink			
			Total ▶			

Stillbirth ☐ Antepartum ☐ Intrapartum

Signature: name/title:

Medication For Baby: Eye Care ☐ Yes ☐ No; Vitamin K ☐ IM ☐ PO ☐ No

Birth Weight: gms. Sex: ☐ Male ☐ Female ☐ Not Known

Postnate Comments:

Anomaly	☐ Yes	☐ No	
Neonate visited	☐ Yes	☐ No	
Neonate passed meconium	☐ Yes	☐ No	
Breast feeding	☐ Yes	☐ No	
Breast fed after delivery	☐ Yes	☐ No	
NICU admission	☐ Yes	☐ No	
Maternal GBS status	☐ Neg	☐ Pos	☐ Not Known
Maternal Hepatitis B Status	☐ Neg	☐ Pos	☐ Not Known
Maternal HIV Status	☐ Neg	☐ Pos	☐ Not Known

Baby stimulated to breathe (yes) (1) / No)

Baby Physician | Baby bed number | Baby Personal Health Number

H50001-126-2 (2008/03)

Appendix II. Supplemental Tables and Figures

Table A2.1. Characteristics of the study population, as reported by the Alberta Perinatal Health Program.

Characteristic	*N (%)*	*Mean (SD)*
Total observations	902 (100.0)	
	14 (1.6)	
Infant characteristics		
Gender		
Female	462 (51.2)	
Male	440 (48.8)	
Birth weight		3358.9g (554.0 g)
Female		3284.3g (513.3 g)
Male		3437.2g (584.2 g)
Low birth weight (<2500 g)	49 (5.4)	2070.2g (560.4 g)
Female	27 (5.8)	2174.4g (447.0 g)
Male	22 (5.0)	1942.3g (662.8 g)
Gestational age		38.7wk (2.1 wk)
Female		38.7wk (2.0 wk)
Male		38.6wk (2.2 wk)
Preterm delivery (<37 wk)	80 (8.9)	34.2wk (3.6 wk)
Female	36 (7.8)	34.2wk (3.4 wk)
Male	44 (10.0)	34.2wk (3.7 wk)
Small for gestational age *(Robertson CM 2002)*	56 (6.2)	
Female	30 (6.5)	
Male	26 (5.9)	
Apgar score		
Apgar (1 minute)		8.1 (1.5)
Apgar (5 minute)		8.9 (0.9)
Apgar <7 (1 minute)	119 (13.2)	
Apgar <7 (5 minute)	14 (1.6)	
Twins	5 (0.6)	
Fetal anomaly		
Suspected anomaly	4 (0.4)	
Observed anomaly	8 (0.9)	
?Down syndrome	1 (0.1)	
Ears lower than canthos	1 (0.1)	
Suspect no right kidney	1 (0.1)	
Tongue tie	1 (0.1)	
Not specified	4 (0.4)	
Stillbirth	1 (0.1)	
Maternal characteristics		
Maternal age		
As recorded on Delivery Record		30.1 (4.8)
As recorded on prenatal screen		30.6 (4.8)
Difference between the two ages		-0.5 (3.0)
Maternal race		
Caucasian	600 (66.5)	
Asian	109 (12.1)	
Hispanic	7 (0.8)	
Black	14 (1.6)	
Semitic	5 (0.6)	
Filipino	12 (1.3)	
First Nation	14 (1.6)	
Oriental	47 (5.2)	
Unknown	94 (10.4)	
Gravida		2.3 (1.3)

Weight		
>91 kg	90 (10.0)	
<45 kg	2 (0.2)	
Height (<152 cm)	17 (1.9)	
Poor weight gain	7 (0.8)	
Previous SGA delivery	8 (0.9)	
Maternal health conditions		
Anemia	0 (0.0)	
Pre-existing diabetes	18 (2.0)	
Gestational diabetes	47 (5.2)	
Heart disease	0 (0.0)	
Hypertension	12 (1.3)	
Pregnancy-induced hypertension (PIH)	42 (4.7)	
Proteinuria	9 (1.0)	
Pre-eclampsia (PIH + proteinuria)	8 (0.9)	
Chronic renal disease	4 (0.4)	
Maternal substance use		
Smoker	99 (11.0)	
Alcohol	5 (0.6)	
Drug-dependent	6 (0.7)	
Antepartum score recorded	902 (100.0)	

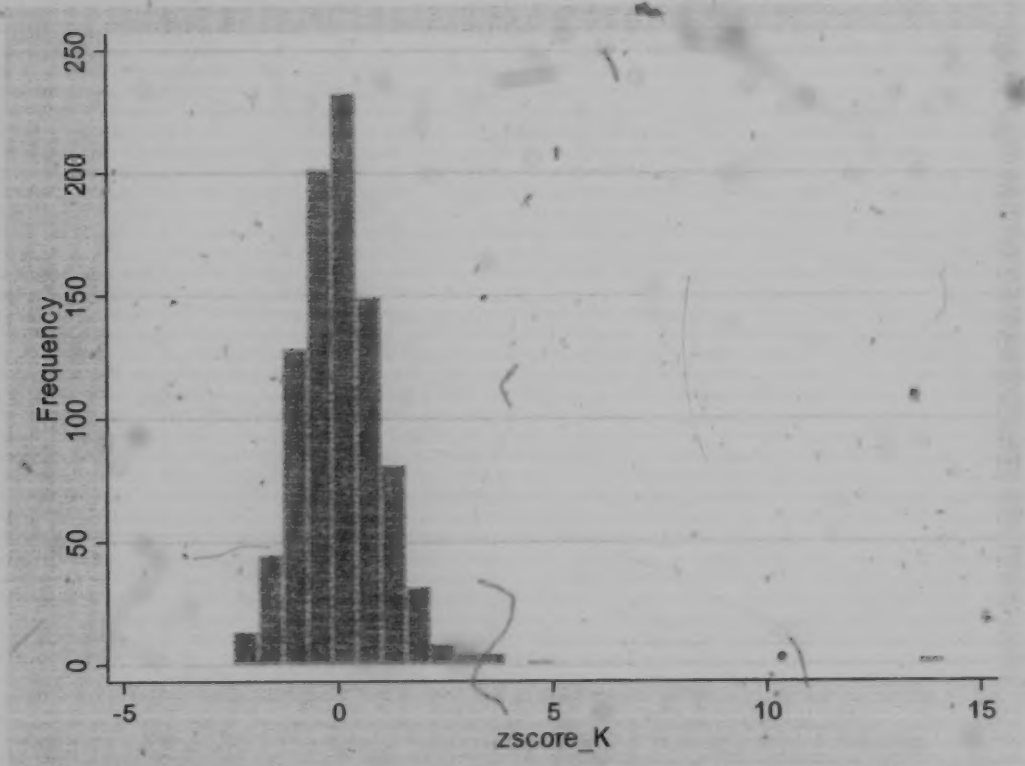


Figure A2.1. Distribution of birth weight z-scores of the entire study population (N=902), calculated according to Kramer *et al.*, 2001.

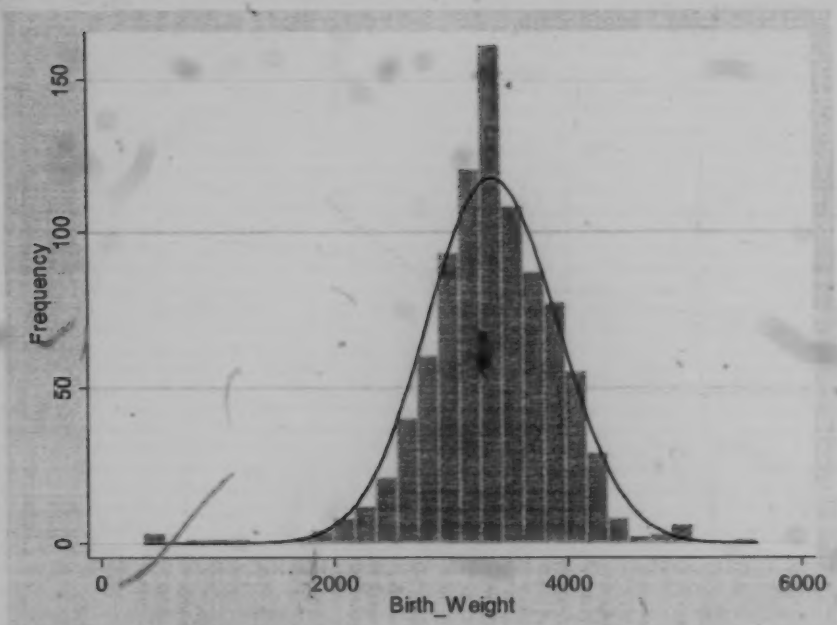


Figure A2.2. Distribution of infant birth weight (grams) of the entire study population (N=902).

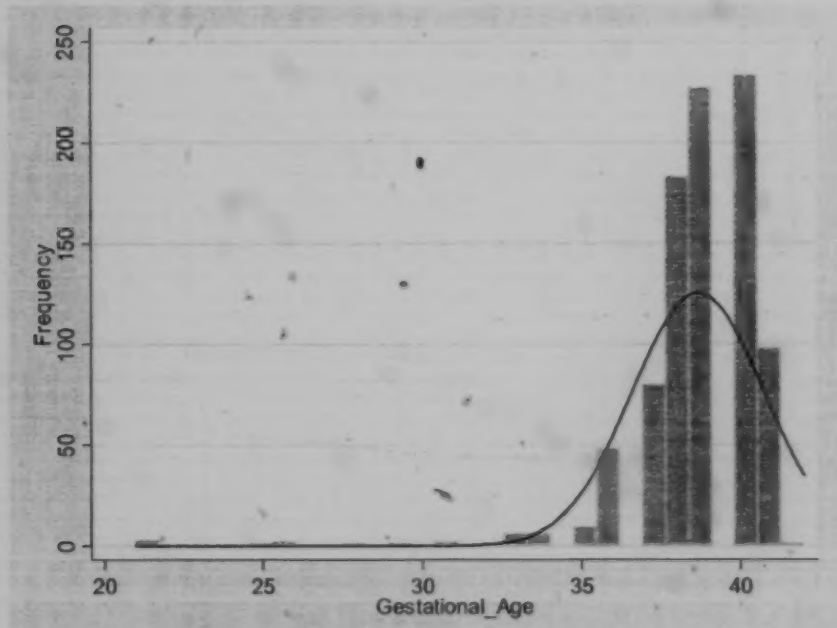


Figure A2.3. Distribution of gestational age (weeks) at delivery of the entire study population (N=902).

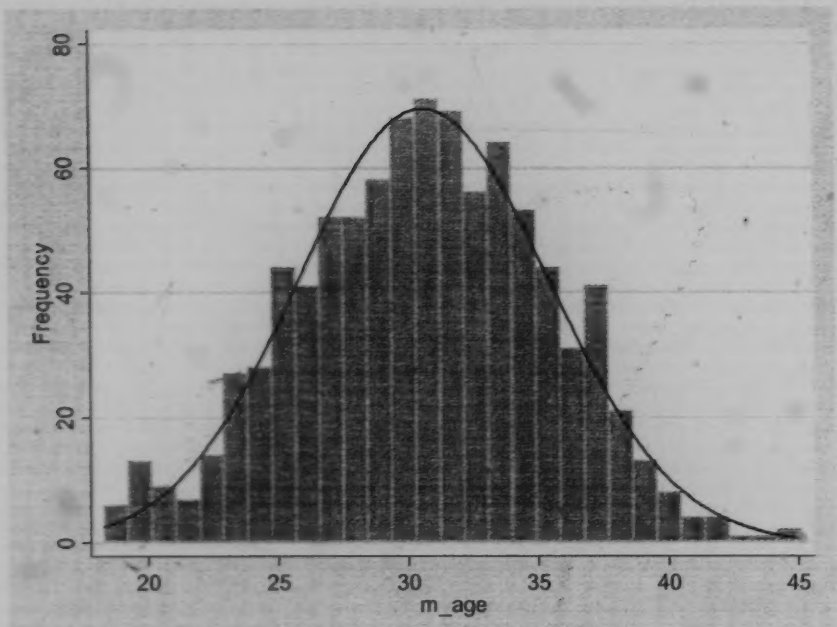


Figure A2.4. Distribution of maternal age (years) of the entire study population (N=902).

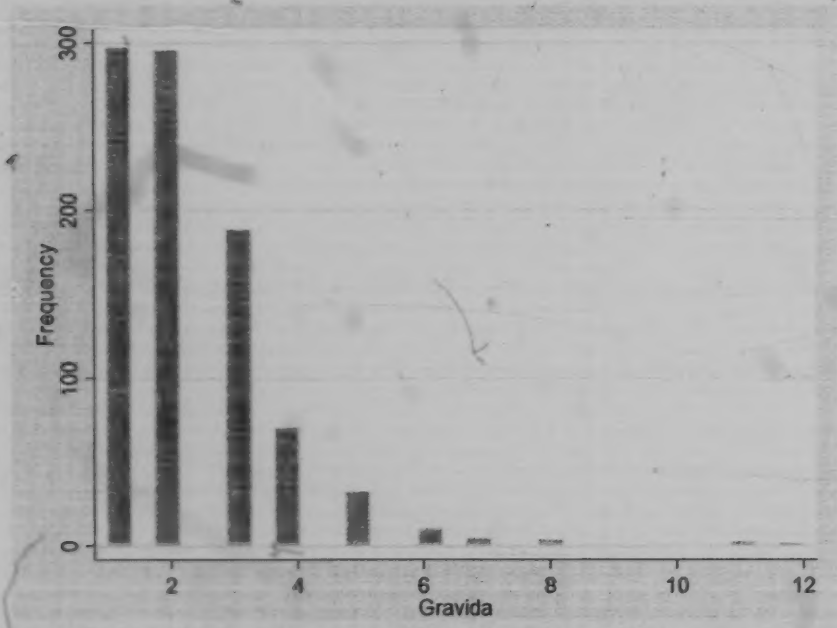


Figure A2.5. Distribution of gravida of the entire study population (N=902).

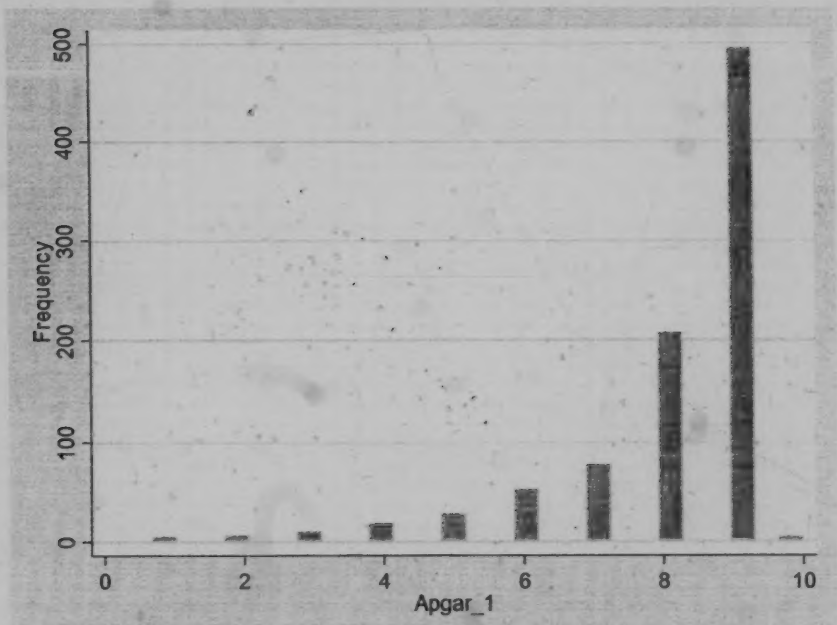


Figure A2.6. Distribution of one-minute Apgar scores of the entire study population (N=902).

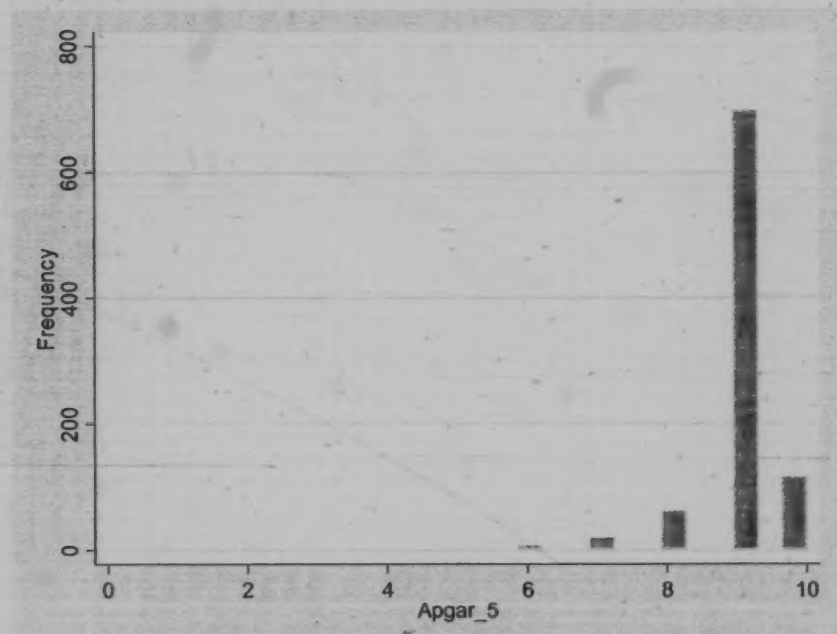


Figure A2.7. Distribution of five-minute Apgar scores of the entire study population (N=902).

Table A2.2. Birth weight z-scores according to infant and maternal variables – linear regression (N=879).

Characteristic	*Kramer*		*APHP*	
	β-coefficient (95% CI)	*p-value*	*β-coefficient (95% CI)*	*p-value*
Gestational age *(25-42 weeks)*	-0.015 (-0.052, 0.022)	0.43	-0.015 (-0.054, 0.024)	0.46
Preterm delivery	0.11 (-0.13, 0.35)	0.37	0.13 (-0.12, 0.38)	0.31
Suspected anomaly	0.24 (-1.08, 1.55)	0.72	0.24 (-1.14, 1.62)	0.74
Maternal age				
Continuous *(18.3-45.1 years)*	0.011 (-0.0020, 0.024)	0.10	0.011 (-0.0023, 0.025)	0.10
Categorical *(N)*				
<20 *(16)*	0.024 (-0.45, 0.50)	0.92	0.032 (-0.47, 0.53)	0.90
20-24 *(98)*	-0.21 (-0.42, 0.0021)	0.05	-0.22 (-0.45, 0.00075)	0.05
25-29 *(267)*	-0.064 (-0.22, 0.088)	0.41	-0.068 (-0.23, 0.092)	0.40
30-34 *(334)*	1.0	-	1.0	-
35-39 *(147)*	-0.024 (-0.21, 0.16)	0.80	-0.027 (-0.22, 0.17)	0.78
≥40 *(17)*	0.052 (-0.41, 0.51)	0.82	0.055 (-0.43, 0.54)	0.82
Maternal race *(N)*				
Caucasian *(589)*	1.0	-	1.0	-
Asian *(103)*	-0.45 (-0.65, -0.25)	0.00	-0.47 (-0.68, -0.27)	0.00
Hispanic *(7)*	-0.29 (-0.99, 0.40)	0.41	-0.30 (-1.03, 0.43)	0.42
Black *(14)*	-0.17 (-0.67, 0.33)	0.50	-0.18 (-0.71, 0.34)	0.49
Semitic *(5)*	-0.39 (-1.22, 0.43)	0.35	-0.42 (-1.29, 0.45)	0.34
Filipino *(11)*	-0.17 (-0.73, 0.39)	0.56	-0.17 (-0.76, 0.42)	0.57
First Nation *(13)*	0.090 (-0.42, 0.61)	0.73	0.085 (-0.46, 0.63)	0.76
Oriental *(47)*	-0.36 (-0.64, -0.080)	0.01	-0.37 (-0.67, -0.080)	0.01
Unknown *(90)*	-0.11 (-0.31, 0.10)	0.32	-0.11 (-0.33, 0.11)	0.33
Gravida				
Continuous *(1-12)*	0.074 (0.028, 0.12)	0.00	0.078 (0.029, 0.13)	0.00
Categorical *(N)*				
1 *(292)*	1.0	-	1.0	-
2 *(288)*	0.025 (-0.13, 0.18)	0.75	0.024 (-0.14, 0.19)	0.77
3 *(180)*	0.21 (0.031, 0.38)	0.02	0.21 (0.028, 0.40)	0.02
≥4 *(119)*	0.25 (0.053, 0.45)	0.01	0.27 (0.054, 0.48)	0.01
Weight >91 kg	0.44 (0.24, 0.65)	0.00	0.46 (0.24, 0.68)	0.00
Weight <45 kg	-0.73 (-2.04, 0.59)	0.28	-0.76 (-2.14, 0.62)	0.28
Height <152 cm	-0.35 (-0.82, 0.12)	0.15	-0.36 (-0.86, 0.13)	0.15
Poor weight gain	-0.49 (-1.20, 0.21)	0.17	-0.51 (-1.25, 0.23)	0.17
Previous SGA delivery	-1.09 (-1.75, -0.44)	0.00	-1.16 (-1.85, -0.47)	0.00
Pre-existing diabetes	0.60 (0.16, 1.04)	0.01	0.64 (0.17, 1.10)	0.01
Gestational diabetes	0.13 (-0.15, 0.40)	0.37	0.13 (-0.16, 0.42)	0.38
Hypertension	0.32 (-0.27, 0.91)	0.29	0.32 (-0.30, 0.94)	0.31
Pregnancy-induced hypertension	-0.039 (-0.33, 0.25)	0.80	-0.041 (-0.35, 0.27)	0.79
Proteinuria	-0.38 (-1.00, 0.24)	0.23	-0.040 (-1.06, 0.25)	0.22
Preeclampsia	-0.51 (-1.17, 0.97)	0.13	-0.55 (-1.24, 0.15)	0.12
Chronic renal disease	-0.099 (-1.17, 0.97)	0.86	-0.13 (-1.26, 1.00)	0.82
Smoker	-0.15 (-0.35, 0.047)	0.14	-0.16 (-0.37, 0.049)	0.13
Alcohol	-0.18 (-1.01, 0.65)	0.68	-0.19 (-1.06, 0.69)	0.67
Drug Dependent	-0.18 (-0.94, 0.58)	0.65	-0.18 (-0.98, 0.62)	0.65
Thyroid hormone status *(N)*				
10^{th}, $<10^{th}$%ile cut-offs				
Euthyroid *(756)*	1.0	-	1.0	-
Hypothyroxinemic *(89)*	0.10 (-0.11, 0.31)	0.35	0.10 (-0.11, 0.32)	0.35
Hypothyroid *(15)*	0.46 (-0.023, 0.94)	0.06	0.49 (-0.022, 1.00)	0.06
Hyperthyroid *(19)*	0.12 (-0.31, 0.55)	0.58	0.13 (-0.32, 0.58)	0.57
Clinical cut-offs				
Euthyroid *(713)*	1.0	-	1.0	-
Hypothyroxinemic *(87)*	0.092 (-0.12, 0.30)	0.39	0.10 (-0.13, 0.32)	0.40
Hypothyroid *(15)*	0.45 (-0.036, 0.94)	0.07	0.48 (-0.036, 0.99)	0.07
Hyperthyroid *(31)*	0.012 (-0.33, 0.35)	0.94	0.20 (-0.34, 0.38)	0.92

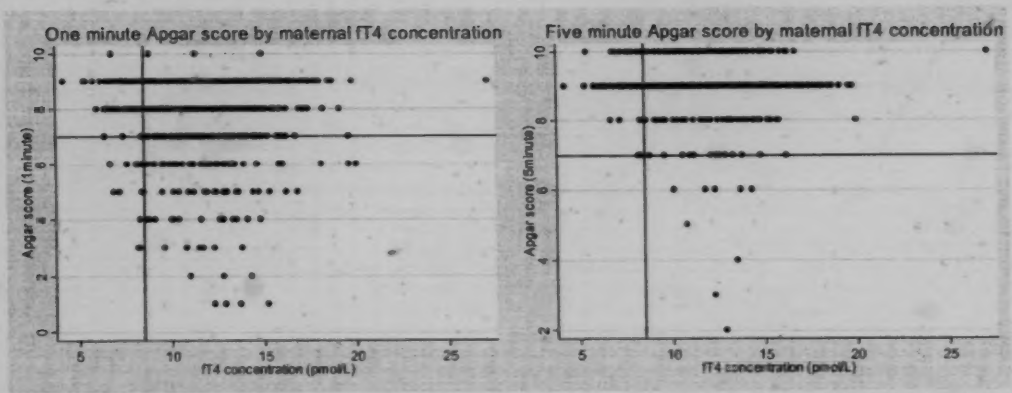

One minute Apgar score by maternal fT4 concentration
Five minute Apgar score by maternal fT4 concentration
Apgar score (1minute)
Apgar score (5minute)
fT4 concentration (pmol/L)
fT4 concentration (pmol/L)

Figure A2.8. One and five minute Apgar scores according to maternal free thyroxine (fT_4) concentration. The vertical line is drawn at the 10^{th} %ile cut-off for fT_4 levels (8.5 pmol/L) and the horizontal line is drawn at an Apgar score of 7.

Table A2.3. Frequency and proportion of low Apgar scores, stratified by maternal thyroid hormone status.

Hypothyroxinemic (N=89)				*Euthyroid (N=756)*				*All study subjects (N=879)*			
Apgar <3		*Apgar <7*		*Apgar <3*		*Apgar <7*		*Apgar <3*		*Apgar <7*	
1 minute	5 minutes	1 minute	5 minutes	1 minute	5 minutes	1 minute	5 minutes	1 minute	5 minutes	1 minute	5 minutes
0 (0.0%)	0 (0.0%)	14 (15.7%)	0 (0.0%)	7 (0.9%)	1 (0.1%)	92 (12.1%)	9 (1.2%)	7 (0.8%)	1 (0.1%)	110 (12.5%)	9 (1.0%)

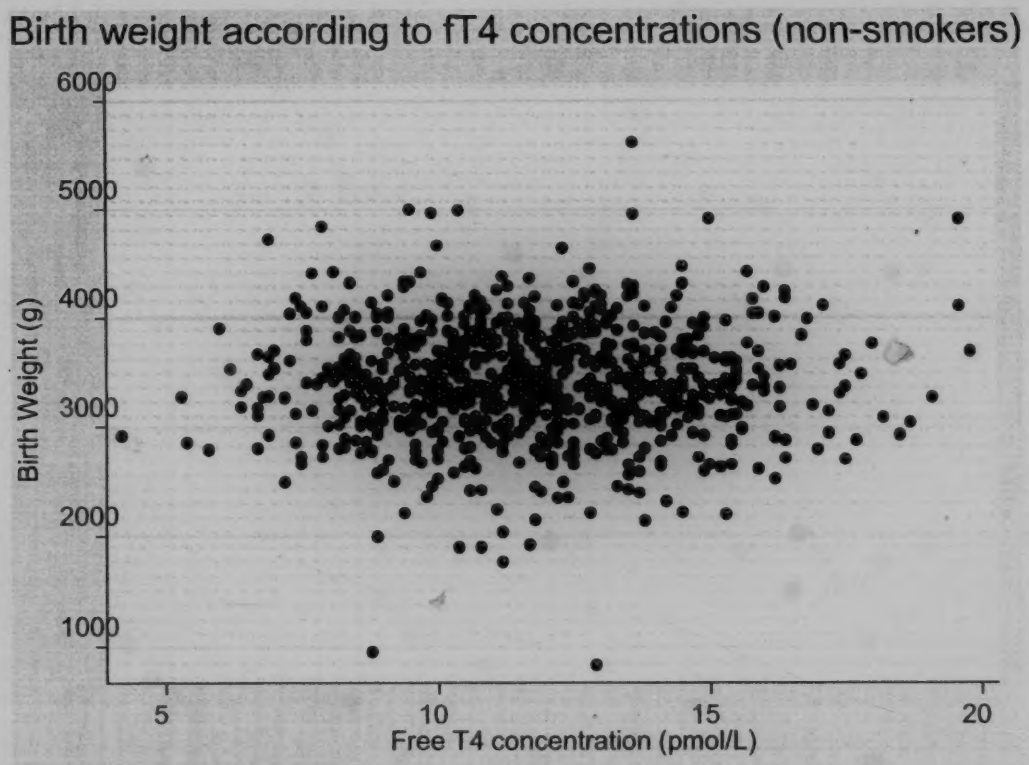


Figure A2.9. Infant birth weight according to maternal free thyroxine (T_4) concentration among non-smokers.

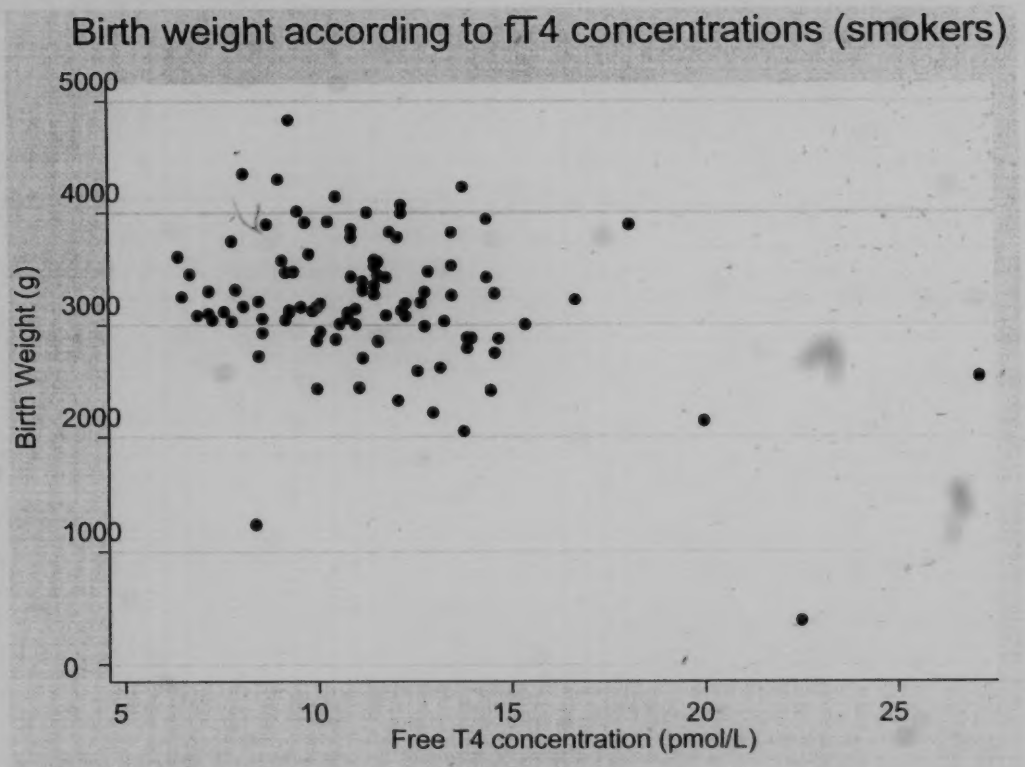


Figure A2.10. Infant birth weight according to maternal free thyroxine (T_4) concentration among smokers.

Table A2.4. Mean birth weight and birth weight z-score, stratified by maternal thyroid hormone levels and smoking status.

	Euthyroid (N)	*Hypothyroxinemic (N)*	*Hypothyroid (N)*	*Hyperthyroid (N)*
Mean birth weight				
Non-smoker	3386.9 g (678)	3448.3 g (75)	3580 g (13)	3392.1 g (17)
Smoker	3223.5 g (80)	3155.0 g (14)	3180 g (2)	2912.5 g (2)
Mean z-score				
Non-smoker	0.090 (678)	0.22 (75)	0.48 (13)	0.19 (17)
Smoker	-0.061 (80)	-0.19 (14)	0.76 (2)	0.10 (2)

Table A2.5. Mean birth weight stratified by thyroid status and infant and maternal variables.
(Mean birth weight in grams, number of study subjects in brackets.)

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Infant variables			
Gender			
Female	3303 (382)	3296 (50)	0.93
Male	3446 (374)	3538 (39)	0.32
Preterm delivery			
No	3436 (701)	3437 (84)	0.99
Yes	2570 (55)	2819 (5)	0.37
Suspected anomaly			
No	3373 (754)	3402 (89)	0.61
Yes	3550 (2)	-	-
Maternal variables			
Maternal age			
<20	3443 (15)	4340 (1)	-
20-24	3335 (90)	2457 (3)	0.01
25-29	3359 (228)	3410 (29)	0.62
30-34	3383 (285)	3483 (40)	0.22
35-39	3381 (124)	3316 (14)	0.66
≥40	3503 (14)	3233 (2)	0.41
Maternal race			
Caucasian	3431 (504)	3444 (68)	0.85
Asian	3173 (88)	3112 (6)	0.77
Hispanic	3240 (6)	3150 (1)	-
Black	3294 (13)	3305 (1)	-
Semitic	3292 (5)	-	-
Filipino	3252 (11)	-	-
First Nation	3270 (10)	2345 (2)	0.16
Oriental	3283 (39)	3411 (5)	0.55
Unknown	3330 (80)	3620 (6)	0.23
Gravida			
1	3357 (260)	3353 (22)	0.98
2	3367 (244)	3279 (33)	0.36
3	3391 (149)	3507 (24)	0.31
≥4	3406 (103)	3664 (10)	0.10
Weight >91 kg			
No	3354 (684)	3420 (79)	0.28
Yes	3554 (72)	3259 (10)	0.12
Weight <45 kg			
No	3374 (754)	3402 (89)	0.63
Yes	3018 (2)	-	-
Height <152 cm			
No	3376 (742)	3402 (88)	0.65
Yes	3018 (2)	3385 (1)	-
Poor weight gain			
No	3377 (750)	3402 (89)	0.66
Yes	2974 (6)	-	-
Previous SGA delivery			
No	3379 (748)	3402 (89)	0.68
Yes	2899 (8)	-	-
Pre-existing diabetes			
No	3379 (742)	3401 (87)	0.71
Yes	3066 (14)	3441 (2)	0.45
Gestational diabetes			
No	3376 (718)	3399 (83)	0.70
Yes	3330 (38)	3441 (6)	0.54

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Hypertension			
No	3375 (746)	3402 (89)	0.63
Yes	3281 (10)	-	-
Pregnancy-induced HTN			
No	3384 (720)	3410 (86)	0.66
Yes	3156 (36)	3172 (3)	0.97
Proteinuria			
No	3379 (747)	3402 (89)	0.69
Yes	2886 (9)	-	-
Preeclampsia			
No	3380 (748)	3402 (89)	0.70
Yes	2769 (8)	-	-
Chronic renal disease			
No	3377 (753)	3402 (89)	0.67
Yes	2418 (3)	-	-
Smoker			
No	3387 (677)	3448 (75)	0.32
Yes	3259 (79)	3155 (14)	0.52
Alcohol use			
No	3373 (752)	3400 (88)	0.64
Yes	3451 (4)	3597 (1)	-
Drug-dependent			
No	3374 (750)	3402 (89)	0.62
Yes	3338 (6)	-	-

* t-test conducted comparing the mean birth weights between euthyroid and hypothyroxinemic groups.

Table A2.6. Mean birth weight z-score stratified by thyroid status and infant and maternal variables.

(Number of study subjects in brackets.)

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Infant variables			
Preterm delivery			
No	0.056 (701)	0.13 (84)	0.51
Yes	0.042 (55)	0.63 (5)	0.16
Suspected anomaly			
No	0.055 (754)	0.16 (89)	0.33
Yes	0.31 (2)	-	-
Maternal variables			
Maternal age			
<20	0.069 (15)	1.30 (1)	-
20-24	-0.098 (90)	-0.51 (3)	0.50
25-29	0.036 (228)	0.21 (29)	0.36
30-34	0.092 (285)	0.20 (40)	0.47
35-39	0.086 (124)	0.027 (14)	0.80
≥40	0.30 (14)	-0.24 (2)	0.44
Maternal race			
Caucasian	0.16 (504)	0.16 (68)	0.96
Asian	-0.34 (88)	-0.48 (6)	0.69
Hispanic	-0.020 (6)	-0.77 (1)	-
Black	-0.041 (13)	0.48 (1)	-
Semitic	-0.23 (5)	-	-
Filipino	-0.0014 (11)	-	-
First Nation	0.29 (10)	0.081 (2)	0.63
Oriental	-0.24 (39)	0.39 (5)	0.08
Unknown	0.017 (80)	0.65 (6)	0.10
Gravida			
1	-0.017 (260)	0.045 (22)	0.77
2	0.0080 (244)	-0.067 (33)	0.65
3	0.18 (149)	0.30 (24)	0.56
≥4	0.17 (103)	0.78 (10)	0.04
Weight >91 kg			
No	0.012 (684)	0.15 (79)	0.20
Yes	0.47 (72)	0.20 (10)	0.42
Weight <45 kg			
No	0.057 (754)	0.16 (89)	0.34
Yes	-0.65 (2)	-	-
Height <152 cm			
No	0.063 (742)	0.15 (88)	0.42
Yes	-0.38 (14)	0.91 (1)	-
Poor weight gain			
No	0.062 (750)	0.16 (89)	0.37
Yes	-0.80 (6)	-	-
Previous SGA delivery			
No	0.067 (748)	0.16 (89)	0.39
Yes	-1.01 (8)	-	-
Pre-existing diabetes			
No	0.049 (742)	0.13 (87)	0.45
Yes	0.37 (14)	1.32 (2)	0.14
Gestational diabetes			
No	0.046 (718)	0.16 (83)	0.30
Yes	0.22 (38)	0.12 (6)	0.78

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Hypertension			
No	0.051 (746)	0.16 (89)	0.32
Yes	0.39 (10)	-	-
Pregnancy-induced HTN			
No	0.059 (720)	0.15 (86)	0.37
Yes	-0.022 (9)	0.21 (3)	0.70
Proteinuria			
No	0.060 (747)	0.16 (89)	0.36
Yes	-0.30 (9)	-	-
Preeclampsia			
No	0.060 (748)	0.16 (89)	0.36
Yes	-0.43 (8)	-	-
Chronic renal disease			
No	0.056 (753)	0.16 (89)	0.34
Yes	-0.022 (3)	-	-
Smoker			
No	0.069 (677)	0.22 (75)	0.18
Yes	-0.061 (79)	-0.19 (14)	0.62
Alcohol use			
No	0.057 (752)	0.15 (88)	0.35
Yes	-0.19 (4)	0.24 (1)	-
Drug-dependent			
No	0.056 (750)	0.16 (89)	0.34
Yes	-0.10 (6)	-	-

* t-test conducted comparing the mean z-scores between euthyroid and hypothyroxinemic groups.

Table A2.7. Proportion of low birth weight infants stratified by thyroid status and infant and maternal variables.

(Proportion of infants born LBW (%), number of study subjects in brackets.)

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Total population	4.9 (37)	1.1 (1)	0.17
Infant variables			
Gender			
Female	2.3 (18)	1.1 (1)	0.71
Male	2.5 (19)	0.0 (0)	0.24
Preterm delivery			
No	1.9 (14)	0.0 (0)	0.38
Yes	3.0 (23)	1.1 (1)	0.64
Suspected anomaly			
No	4.9 (37)	1.1 (1)	0.17
Yes	0.0 (0)	-	-
Maternal variables			
Maternal age			
<20	0.1 (1)	0.0 (0)	1.00
20-24	0.9 (7)	1.1 (1)	0.24
25-29	1.5 (11)	0.0 (0)	0.62
30-34	1.9 (14)	0.0 (0)	0.23
35-39	0.5 (4)	0.0 (0)	1.00
≥40	0.0 (0)	0.0 (0)	-
Maternal race			
Caucasian	2.6 (20)	0.0 (0)	0.15
Asian	0.8 (6)	0.0 (0)	1.00
Hispanic	0.0 (0)	0.0 (0)	-
Black	0.1 (1)	0.0 (0)	1.00
Semitic	0.0 (0)	-	-
Filipino	0.1 (1)	-	-
First Nation	0.1 (1)	1.1 (1)	0.32
Oriental	0.3 (2)	0.0 (0)	1.00
Unknown	0.8 (6)	0.0 (0)	1.00
Gravida			
1	1.6 (12)	1.1 (1)	1.00
2	1.6 (12)	0.0 (0)	0.37
3	1.2 (9)	0.0 (0)	0.61
≥4	0.5 (4)	0.0 (0)	1.00
Weight >91 kg			
No	4.8 (36)	0.0 (0)	0.04
Yes	0.1 (1)	1.1 (1)	0.23
Weight <45 kg			
No	4.9 (37)	1.1 (1)	0.17
Yes	0.0 (0)	-	-
Height <152 cm			
No	4.8 (36)	1.1 (1)	0.17
Yes	0.1 (1)	0.0 (0)	1.00
Poor weight gain			
No	4.8 (36)	1.1 (1)	0.17
Yes	0.1 (1)	-	-
Previous SGA delivery			
No	4.6 (35)	1.1 (1)	0.17
Yes	0.3 (2)	-	-
Pre-existing diabetes			
No	4.4 (33)	1.1 (1)	0.25
Yes	0.5 (4)	0.0 (0)	1.00
Gestational diabetes			
No	4.8 (36)	1.1 (1)	0.17
Yes	0.1 (1)	0.0 (0)	1.00

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Hypertension			
No	4.6 (35)	1.1 (1)	0.17
Yes	0.3 (2)	-	-
Pregnancy-induced HTN			
No	4.2 (32)	1.1 (1)	0.24
Yes	0.6 (5)	0.0 (0)	1.00
Proteinuria			
No	4.5 (34)	1.1 (1)	0.16
Yes	0.4 (3)	-	-
Preeclampsia			
No	4.5 (34)	1.1 (1)	0.16
Yes	0.4 (3)	-	-
Chronic renal disease			
No	4.6 (35)	1.1 (1)	0.17
Yes	0.3 (2)	-	-
Smoker			
No	4.0 (30)	0.0 (0)	0.06
Yes	0.9 (7)	1.1 (1)	1.00
Alcohol use			
No	4.9 (37)	1.1 (1)	0.17
Yes	0.0 (0)	0.0 (0)	-
Drug-dependent			
No	4.9 (37)	1.1 (1)	0.17
Yes	0.0 (0)	-	-

* t-test conducted comparing the mean birth weights between euthyroid and hypothyroxinemic groups.

Table A2.8. Proportion of small for gestational age infants stratified by thyroid status and infant and maternal variables.

(Proportion of infants born SGA (%),, number of study subjects in brackets.)

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Total population	6.7 (51)	1.1 (1)	0.03
Infant variables			
Suspected anomaly			
No	6.7 (51)	1.1 (1)	0.03
Yes	0.0 (0)	-	-
Maternal variables			
Maternal age			
<20	0.4 (3)	0.0 (0)	1.00
20-24	1.6 (12)	0.0 (0)	1.00
25-29	2.1 (16)	0.0 (0)	0.23
30-34	1.7 (13)	0.0 (0)	0.38
35-39	0.9 (7)	0.0 (0)	1.00
≥40	0.0 (0)	1.1 (1.0)	0.13
Maternal race			
Caucasian	3.3 (25)	1.1 (1)	0.16
Asian	1.6 (12)	0.0 (0)	0.43
Hispanic	0.1 (1)	0.0 (0)	0.86
Black	2.6 (2)	0.0 (0)	1.00
Semitic	0.1 (1)	-	-
Filipino	0.1 (1)	-	-
First Nation	0.0 (0)	0.0 (0)	-
Oriental	0.4 (3)	0.0 (0)	1.00
Unknown	0.8 (6)	0.0 (0)	1.00
Gravida			
1	2.9 (22)	1.1 (1)	1.00
2	2.0 (15)	0.0 (0)	0.23
3	1.3 (10)	0.0 (0)	0.36
≥4	0.5 (4)	0.0 (0)	1.00
Weight >91 kg			
No	6.5 (49)	1.1 (1)	0.05
Yes	0.3 (2)	0.0 (0)	1.00
Weight <45 kg			
No	6.6 (50)	1.1 (1)	0.03
Yes	0.1 (1)	-	-
Height <152 cm			
No	6.3 (48)	1.1 (1)	0.05
Yes	0.4 (3)	0.0 (0)	1.00
Poor weight gain			
No	6.5 (49)	1.1 (1)	0.05
Yes	0.3 (2)	-	-
Previous SGA delivery			
No	6.5 (49)	1.1 (1)	0.03
Yes	0.3 (2)	-	-
Pre-existing diabetes			
No	6.7 (51)	1.1 (1)	0.03
Yes	0.0 (0)	0.0 (0)	-
Gestational diabetes			
No	6.6 (50)	1.1 (1)	0.05
Yes	0.1 (1)	0.0 (0)	1.00

Characteristic	*Euthyroid (N=756)*	*Hypothyroxinemic (N=89)*	*p-value**
Hypertension			
No	6.7 (51)	1.1 (1)	0.03
Yes	0.0 (50)	-	-
Pregnancy-induced HTN			
No	6.3 (48)	1.1 (1)	0.05
Yes	0.4 (3)	0.0 (0)	1.00
Proteinuria			
No	6.5 (49)	1.1 (1)	0.03
Yes	0.3 (2)	-	-
Preeclampsia			
No	6.5 (49)	1.1 (1)	0.03
Yes	0.3 (2)	-	-
Chronic renal disease			
No	6.7 (51)	1.1 (1)	0.03
Yes	0.0 (0)	-	-
Smoker			
No	5.6 (42)	1.1 (1)	0.11
Yes	1.2 (9)	0.0 (0)	0.35
Alcohol use			
No	6.7 (51)	1.1 (1)	0.03
Yes	0.0 (0)	0.0 (0)	-
Drug-dependent			
No	6.7 (51)	1.1 (1)	0.03
Yes	0.0 (0)	-	-

* t-test conducted comparing the mean birth weights between euthyroid and hypothyroxinemic groups.

Appendix III. Stata Modeling Codes

Dataset: thyroid_final_reduced.dta

Variable coding:

SGA: Small for gestational age

zscore_K: Birth weight z-score (Kramer *et al.*, 2001)

apgar5_7: Five minute Apgar score <7

htx: Maternal hypothyroxinemia (TSH 0.15-4.0 mU/L; free T_4 ≤8.5 pmol/L)

hypo: Maternal hypothyroidism (TSH >4.0 mU/L)

hyper: Maternal hyperthyroidism (TSH <0.2 mU/L)

fT4: Free thyroxine concentration

TSH: Thyroid-stimulating hormone concentration

fT4_ga_day: Free thyroxine concentration * gestational age at prenatal screen (days)

susp_anomaly1: Suspected fetal anomaly

m_age: Maternal age in years

m_race: Self-reported maternal race

gr: Gravida

wt91_1: Maternal weight >91 kg

wt45_1: Maternal weight <45 kg

height1: Maternal height <152 cm

pwg1: Poor maternal weight gain during pregnancy

prev_sga1: Previous small for gestational age delivery

pre_diab1: Pre-existing maternal diabetes

gest_diab1: Gestational diabetes

HTN1: Maternal hypertension

preeclampsia: Pregnancy-induced hypertension with proteinuria

chr_renal1: Chronic maternal renal disease

smoker1: Self-reported smoking status

alcohol1: Maternal consumption of ≥ 3 drinks or ≥ 1 drink per day

drug_dependent1: Maternal drug dependency

year2: Maternal age squared

sex: Infant gender

gestational_age: Gestational age at delivery (weeks)

gage2: Gestational age at delivery (weeks) squared

Small for gestational age:

xi: glm SGA i.htx i.hypo i.hyper i.susp_anomaly1 m_age i.m_race i.gr i.wt91_1 i.wt45_1 i.height1 i.pwg1 i.prev_sga1 i.pre_diab1 i.gest_diab1 i.HTN1 i.preeclampsia i.chr_renal1 i.smoker1 i.alcohol1 i.drug_dependent1 year2, fam(poisson) link(log) nolog robust eform

xi: glm SGA fT4 TSH fT4_ga_day i.susp_anomaly1 m_age i.m_race i.gr i.wt91_1 i.wt45_1 i.height1 i.pwg1 i.prev_sga1 i.pre_diab1 i.gest_diab1 i.HTN1 i.preeclampsia i.chr_renal1 i.smoker1 i.alcohol1 i.drug_dependent1 year2, fam(poisson) link(log) nolog robust eform

Z-score:

xi: regress zscore_K i.htx i.hypo i.hyper i.susp_anomaly1 m_age year2 i.m_race i.gr i.wt91_1 i.wt45_1 i.height1 i.pwg1 i.prev_sga1 i.pre_diab1 i.gest_diab1 i.HTN1 i.preeclampsia i.chr_renal1 i.smoker1 i.alcohol1 i.drug_dependent1

xi: regress zscore_K fT4 TSH fT4_ga_day i.susp_anomaly1 m_age i.m_race i.gr i.wt91_1 i.wt45_1 i.height1 i.pwg1 i.prev_sga1 i.pre_diab1 i.gest_diab1 i.HTN1 i.preeclampsia i.chr_renal1 i.smoker1 i.alcohol1 i.drug_dependent1 year2

Apgar:

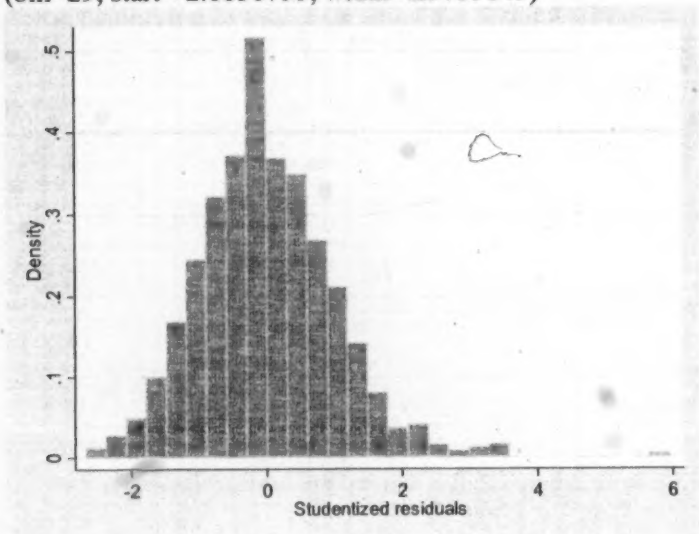
xi: glm apgar5_7 fT4 TSH fT4_ga_day i.sex gestational_age i.susp_anomaly1 m_age i.m_race i.gr i.wt91_1 i.wt45_1 i.height1 i.pwg1 i.prev_sga1 i.pre_diab1 i.gest_diab1 i.HTN1 i.preeclampsia i.chr_renal1 i.smoker1 i.alcohol1 i.drug_dependent1 year2 gage2, fam(poisson) link(log) nolog robust eform

Appendix IV. Regression Diagnostics

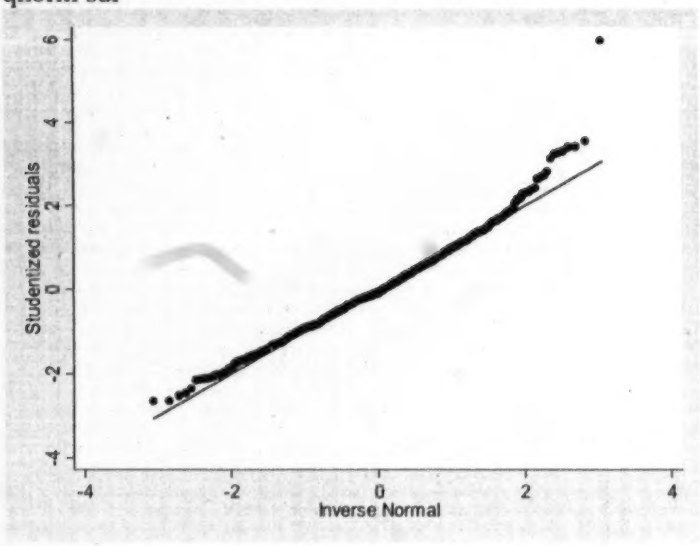
Dependent variable: Birth weight z-score

Independent variables: Hypothyroxinemia, hypothyroidism, hyperthyroidism, suspected fetal anomaly, maternal age (years), maternal age squared (years), maternal race, gravida, maternal weight >91 kg, maternal weight <45 kg, maternal height <152 cm, poor maternal weight gain, previous SGA delivery, pre-existing diabetes, gestational diabetes, hypertension, preeclampsia, chronic renal disease, smoking status, alcohol consumption, drug dependence

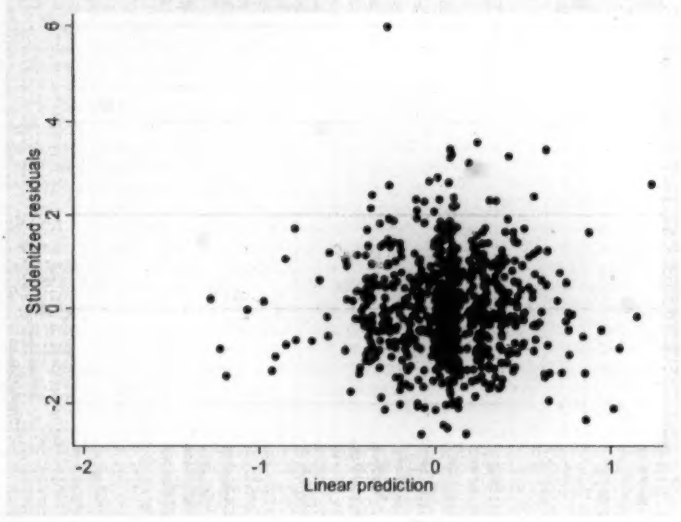
```
quietly xi: regress zscore_K i.htx i.hypo i.hyper i.susp_anomaly1 m_age year2 i.m_race
i.gr i.wt91_1 i.wt45_1 i.height1 i.pwg1 i.prev_sga1 i.pre_diab1 i.gest_diab1 i.HTN1
i.preeclampsia i.chr_renal1 i.smoker1 i.alcohol1 i.drug_dependent1

predict pred, xb
predict sdr, rstudent

hist sdr
(bin=29, start=-2.6636755, width=.29759849)
```



qnorm sdr



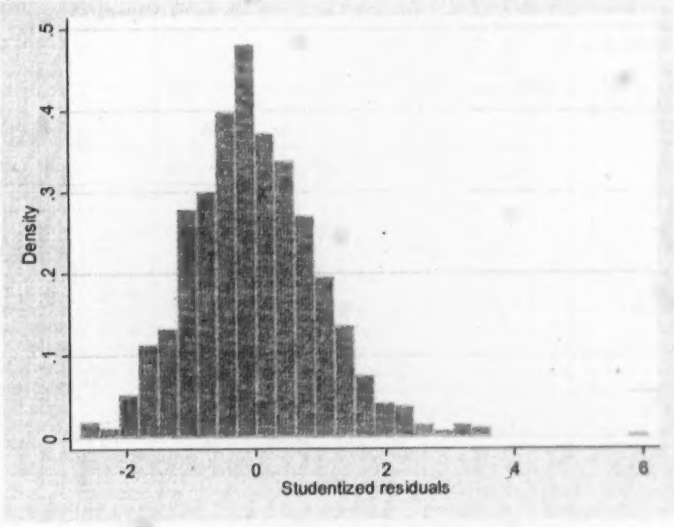
twoway (scatter sdr pred)



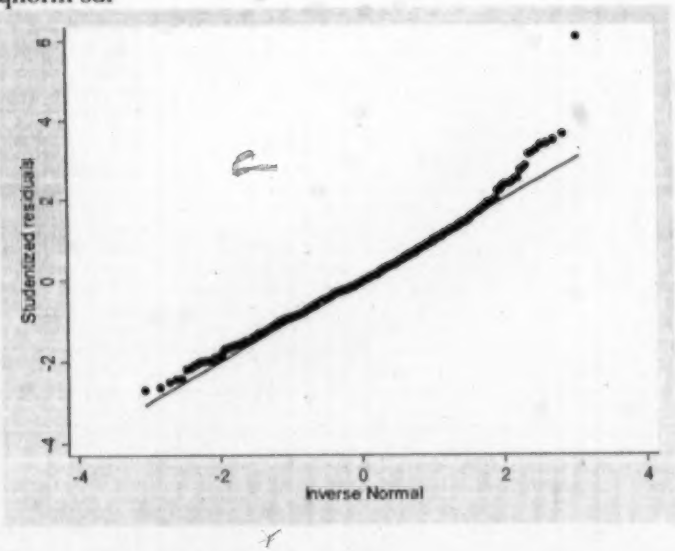
Dependent variable: Birth weight z-score

Independent variables: Free T_4 concentration, TSH concentration, free T_4 * gestational age at prenatal screen, suspected fetal anomaly, maternal age (years), maternal race, gravida, maternal weight >91 kg, maternal weight <45 kg, maternal height <152 cm, poor maternal weight gain, previous SGA delivery, pre-existing diabetes, gestational diabetes, hypertension, preeclampsia, chronic renal disease, smoking status, alcohol consumption, drug dependence, maternal age squared (years)

```
quietly xi: regress zscore_K fT4 TSH fT4_ga_day i.susp_anomaly1 m_age i.m_race i.gr
i.wt91_1 i.wt45_1 i.height1 i.pwg1 i.prev_sga1 i.pre_diab1 i.gest_diab1 i.HTN1
i.preeclampsia i.chr_renal1 i.smoker1 i.alcohol1 i.drug_dependent1 year2

predict pred, xb
predict sdr, rstudent

hist sdr
(bin=29, start=-2.7123129, width=.3031273)
```



qnorm sdr



twoway (scatter sdr pred)